免疫生物学
シンポジウム 4

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Introductory remarks : 胸腺依存リンパ球の動態

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Thymus-dependent Lymphocytes in Lymph Node and Spleen. MASAO HANAOKA (Department of Pathology, Inst. for Virus Research, Kyoto University)

ABSTRACT : So-called thymus-dependent lymphocytes (TDL) are distributed as a dense mass of small lymphocytes around the postcapillary vein in the paracortical area of lymph node and in the periarteriolar region of the white pulp of spleen. TDL almost compledtely disappeared from lymph node and spleen of mice injected with lymphocytosis-promoting factor which was obtained from cultures of B. pertussis. This finding suggests that TDL belong to the circulating lymphocyte. TDL were also almost completely absent from lymph nodes and spleens of thymectomized, X-irradiated rabbits. One to two weeks after the intraperitoneal transplantation of autologous unirradiated thymus into appendictomized, thymectomized and X-irradiated rabbits, TDL were found massively around the PCV of lymph node and in the periarterioar region of the white pulp of spleen whithout the recovery of the germinal center and lymphocytes in other areas of both lymphoid organs. In appendictomized, X-irradiated rabbits, sometimes TDL proliferated around the PCV of lymph nodes resembling the proliferating pattern of blast-trnasfromation of small lymphocytes stimulated in vitro by PHA, PPD, etc. IgG, IgA, IgM, and anti-BγG were not contained in TDL in lymph node and spleen of rabbits immunized with BγG. From above findings, the role of TDL in cell mediated immunity is discussed.

Cell-mediated immunityに重要な役割をもつ胸腺を除去したとき，末梢リンパ組組から消失する〝胸腺依存リンパ球の動態について，マウス及びウサギを用いて，組織学的に検索した．

百日咳菌培養液上清から得られたリンパ球動員因子（LPF）（岩佐氏法による）をマウスに接種すると，リンパ球の再循環を封じられた結果，リンパ節皮質深部から殆どのリンパ球が姿を消して終う．この部位は副皮質（PCA）と呼ばれており，その主構成リンパ球は循環リンパ球である．また，リンパ球性脈絡髄膜炎ウイルスを接種4日後のマウスリンパ節をみると，副皮質の後毛細血管静脈周域，皮質周辺部及び髄索からはリンパ球が消失していないが，副皮質の周辺部に於て，選択的にリンパ球が障害され姿を消している．これらの所見から，リンパ節皮質は皮質周域と副皮質に分れるが，リンパ球の動きからみて，副皮質内の後毛細血管静脈周域に密に介在しているリンパ球群は，その他の副皮質内のリンパ球と異った態度を示すことが分る．そこで，新生マウスの胸腺を剔出し，1月後のリンパ節をみると，よく知られている如く，副皮質全域から大半のリンパ球が姿を消している．故に，副皮質が胸腺依存域と呼ばれるわけである．尚，これらのリンパ節で，副皮質の周辺部に，しばしば細網細胞からリンパ芽球への移行像が見られる．

この副皮質即ち胸腺依存域について詳しく観察するため，生後4週のウサギから，胸腺または虫垂を剔出，X線照射，抗原注射を行ったのち，両群のリンパ節を比較観察した．胸腺剔出群では，皮質周域のリンパ球は残っているが，副皮質及び髄索には殆どリンパ球はみられない．他方，虫垂

剔出群においては，副皮質後毛細管静脈周域に，リンパ球が密に集っているが，副皮質の他の部位及び髄索には殆どリンパ球はみられない．従って，胸腺の存在に特異的に依存しているリンパ球は，この後毛細管周のリンパ球集団であるといえよう．故に，これらのリンパ球を“胸腺依存リンパ球”と呼ぶことにする．このことは，新生期胸腺または出産剔出，X線照射ウサギのリンパ節所見の結果と一致する．さらに，生後3月前後のウサギの胸腺および出産を剔出し，直にウサギにX線を照射し，自己の非照射胸腺あるいは出産を腹腔内に移植，抗原注射10～14日後のリンパ節を観察した．胸腺・出産剔出，X線照射・抗原注射のみの対照群では，リンパ球の再生が殆どなく，皮質・髄索とも細網細胞で占められ，胚中心の形成もない．しかし，出産移植群では，胚中心の形成と細網細胞・リンパ胚球の増生がある．小リンパ球は殆どみられない．これに反し，胸腺移植群では，後毛細管静脈周域に，密に小リンパ球が集り島状となっている．胚中心形成，細網細胞・リンパ胚球の増生はみられない．同様の対照的な所見は，脾においてもみられ，胸腺移植群では，中心動脈周囲を中心に多数の小リンパ球が，胚中心形成を伴うことなく，集っている．このことから，リンパ節副皮質の後毛細管静脈周および脾中心動脈周のリンパ球が，胸腺依存性であることがさらに明らかになった．これらのリンパ球は殆どすべて中・小リンパ球である．ところが，前述の出産剔出・X線照射ウサギのリンパ節後毛細管静脈周域に，無処置あるいは免疫ウサギリンパ節のそれらより，リンパ球がより多く集団をなしている例がしばしばみられた．この部分を詳しくみると，細網細胞の増生はなく，主に円型遊離型の大・小リンパ球が混在している．とくに，中・小型のリンパ球の核内に，1個の核仁が明瞭にみられるものが多く，大型リンパ球の核は，巨大核仁1個をもったものか，小核仁2～3個を備えたものが見出される．所が，in vitro において，PHAによるリンパ球の若返り過程を連続的に観察すると，まず小リンパ球内に1個の核仁が明瞭となり，細胞質RNAが増加するとともに，そのままの形で，^{3}H-TdRをとりこむことなく肥大し，2～3日後巨大核仁を1個もった大型リンパ球になって，はじめて分裂し，核仁が小型になって分散してゆく．前記後毛細管静脈周の増生リンパ球も，この過程をたどって若返り，増殖しているものと考えられる．また，BCG免疫ウサギリンパ節において，この後毛細管静脈周の胸腺依存リンパ球は，IG，IA，IMおよび抗-BCGのいずれをもっていない．これらの点から，後毛細管静脈周域のリンパ球は，PPD，同種抗原などで若返り，細胞障害因子を出し，cell-mediated immunityに関与するリンパ球と考えられる．

鳥類の胸腺とファブリシウス嚢の機能

佐藤孝二（名大・畜・畜産）

Immunological Functions of the Thymus and the Bursa of Fabricius. KOJI SATO (Department of Animal Physiology, Nagoya University)

ABSTRACT: There are two functionally different populations of immunologically competent lymphoid cells; the thymus-dependent and the bursa-dependent populations(Figure 1). The thymus-dependent lymphoid cells are generally small and responsible for cell-mediate immune response, such as homograft rejection and delayed hypersensitivity. The bursa-dependent lymphoid cells, consist of germinal centers in the spleen, are comparatively larger than those of the thymus-dependent cells and relate to plasmacytic cells, which is responsible for the production of immunoglobulin.

Several differences between these two cell populations have been reported; cell size, ribosome, enzyme activity and response to chemical agents (Table 1). Significant prolongation of homografts were observed only in corticosterone treated chicks. However, antibody response was suppressed by cortisone and hydrocrotisone.

Evidence of a direct relation between the thymus and the bursa of Fabricius is obtained by using autoradiographic method. H^3-Thymidine in situ labelled cells emigrated from the thymus to the bursa and or from the bursa to the thymus (Table 2).

胸腺とファブリシウス嚢の免疫的機能に関しては，第1回（小林，山口）および第2回（山口）シンポジウムにおいて，すでに報告されている。ここでは　その後における研究の進展について述べることにする。

胸腺は，免疫的機能の発達に関し中枢的役割を演ずるといわれているが，鳥類では，胸腺とともに，もう一つの中枢として，ファブリシウス嚢が総排泄腔の背壁に存在し，両者の間に一種の機能的分担がみられる。すなわち，胸腺は移植免疫の発達に，ファブリシウス嚢は，体液性抗体の産生に関与し，末梢のリンパ系組織も，その発達が胸腺に依存する系と，ファブリシウス嚢に依存する系とに分けられる。以上，両者の関係を模式的に示してみると次の様になる。

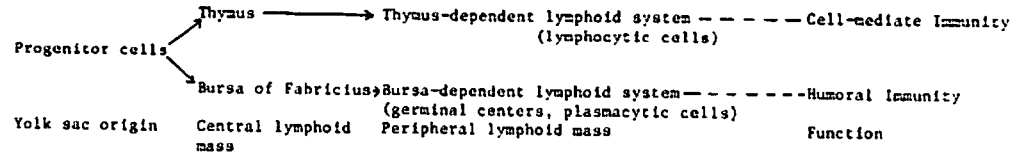

Figure 1. Diagramatic scheme of two functionally different lymphoid systems.

ファブリシウス嚢と抗体産生能との関連については，多くの検討が行われている。ファブリシウス嚢は，IgM様の蛋白質を含有するにも拘らず，

抗体を産生する能力を欠く。ファブリシウス嚢は，脾臓とう末梢にリンパ系細胞を供給し，抗体産生能の成熟化を支配すると考えられている。ホルモン様物質の存在は，実験の再現性に乏しく，将来の検討にまたねばならない。

一方，胸腺に関しては，胸腺を摘除することにより，ヒナは移植皮膚をうけいれ，ツベルクリン反応，アレルギー性脳脊髄膜炎とう遅延型過敏反応が低下することはよく知られている。しかし，ファブリシウス嚢あるいはマウスにおける胸腺の場合ほどは，実験が進められておらず，いかなる機作で，胸腺は移植免疫の発達に関与するか，全く不明といっても過言ではない。胸腺を除去すると末梢リンパ系組織から小型のリンパ球が減少するので，おそらく，胸腺の機能は，胸腺で活潑に造成されるリンパ系細胞を通じて行われると想像されている。

Lymphoid system	cell size	RNA	Enzyme[1]	PHA[2]	Testosterone	Corticosterone	Cortisone
Thymus	3.5-4.0μ	low	high	reactive	+[3]	+++	++
Bursa of Fabricius	4.0-8.0	high	low	none	+++	+	+

1)Lysolecitithine,transferase, 2)Phytohaemagglutinin, 3)Atrophy

Table 1. Comparative data of two functionary different lymphoid system.

従って，ニワトリでは，直接的あるいは間接的に免疫反応に関与するリンパ系細胞に，それぞれの役割を異にした2つの細胞集団が存在することになる。胸腺およびファブリシウス嚢のリンパ系細胞の大部分は，いわゆる小リンパ球と呼ばれる形態的特徴を有しているが，第1表に示す様に，質的にも，両者は異った細胞である。この様な違いがそれぞれの免疫的役割の違いを投影するのであるかどうか。両者の差を究明に追求することは，多くの謎に包まれている免疫的機構を解明する手がかりの一つともなろう。

しかしながら，機能分担があるとはいえ，胸腺，ファブリシウス嚢両者が相互に影響を及ぼしている可能性もある。例えば，抗体産生能は，胸腺除去により，必ずしも抑制はされないが，値の変動が著しくなる。あるいは，ニワトリの自己免疫疾患である甲状腺炎は，ファブリシウス嚢依存のリンパ系細胞の浸じゅんを特徴とするが，胸腺を摘除すると，その浸じゅんが増強するという報告もある。胸腺を除去したヒナのファブリシウス嚢沪胞では，リンパ系細胞が減少する。

この様な実験結果は，いずれも，胸腺－ファブリシウス嚢相互の影響を示唆するものであり，骨ずいを含めた3者間における細胞相互の関係は，

免疫機構と関連させて，将来追求すべきテーマと思われる。

以下に，H^3-チミジンで標識した細胞が，胸腺あるいは，ファブリシウス嚢に移行し，出現する結果を示した。

Chick No.	Dose	Thymus	Spleen	Cecum Tonsile	Bursa of Fabricius	Bone Marrow
Intra-Thymic Injection of H^3-Thymidine						
4 hours after						
525	24μC	+	+	-	+	-
526	24	+	+	-	-	-
24 hours after						
519	14	+	-	-	+	+
524	24	+	+	+	+	-
48 hours after						
520	24	+	+	+	+	-
523	24	+	+	+	+	-
Intra-bursal Injection of H^3-Thymidine						
527	12μC	+	+	-	+	+

+Presence of silver grain

Table 2. Emigration of H^3-labelled cells.

無菌動物のリンパ系

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The lymphatic system of germfree animals. YUTAKA UNO (Germfree Life Research Laboratory, Nagoya University School of Medicine)

A germfree animal may be defined as one that has been born and raised under sterile conditions so it has had no contact with bacteria and thus has never experienced any bacterial invasion of its tissues. It is a matter of great interest to determine the effects of such a germfree life on lymphatic tissues.

Guinea pigs of the Gifu uniform strain and rats of the Fisher strain were employed. Both germfree and conventional animals were kept in the same environmental conditions.

The experimental results as follows.

I made a preliminary investigation on many conventional animals, ranging in deveropment from fetus to adult, in order to discover the essential status of the lymphatic apparatus in the nasal cavity.

In the 40-day-old conventional guinea pig, well-developed lymphatic tissue was found in the nasal cavity, wherin not only solid secondary nodules but also centered secondary nodules were formed. In germfree guinea pigs of the same age, similar lymphatic tissue and occasional solid secondary nodules were found at the nasal floor, but no clear-centered nodules.

As result of the examination of the lymph node of germfree animals, I can say that the lymph node in germfree animals develop more poorly than do those in natural guinea pigs.

The thymus of the germfree rat was compared with that of the conventional rat, using histological, histochemical, and tissue quantitation methods. The lymphocytes in the thymic cortex were apparently more concentrated in the conventional than in the germfree rats. Plasma cells were present in the conventional rat, while they were lacking in the thymus of the germfree rat.

無菌動物は、体表及び腸管内のいかなる所にも微生物を欠き、外来感染をうけないので、生体の反応は少いといえる。無菌動物の特性の一つに盲腸の拡張があげられるが、その原因は不明である。全身リンパ系は発達が悪く、特に腸間膜リンパ節、及び盲腸根部リンパ節は自然動物に較べて非常に小さい。形態的には follicle は全く発達せず、一般に皮質の小形リンパ球の減少が見られ、充実性と胞小節の出現が少なく、時にはこれをなくすこともある。合成飼料 MI-2 にて育てたモルモットの腸間膜リンパ節の重量の体重比 (mg/g) の平均は 1～30 日の年令層において、自然動物では 0.684 であったのに対して、無菌動物では 0.17 であった。又 61－81 年

左膝において、自然動物の1.12に対して、無菌動物では0.298であり、その間に明らかな有意差の差が見られた。

上頸洞リンパ装置も同様に無菌動物において、発達が悪い。自然ラットでは、生後40日で上頸リンパ装置の発育が良好で二次小節の形成があり、反応中枢と認めることが多い。無菌動物においては、同年令においてはなはだ貧弱な像でとどまり、完全性二次小節の形成すら見られない。

次に無菌動物を自然界に暴露すると、腸管特に盲腸及び大腸の部に強い炎性反応が見られ、リンパ節腫脹や脾腫が見られる。特にpeyer板に壊死崩壊が見られ、その壊痕底には組織内細菌塊色にそまる無数の細菌が見られ、それらの細菌は壊痕底に露出した毛細管壁に接するように侵入しているところと観察される。

リンパ装置の低形成に関連して、胸腺組織の発育状況が問題となるが、胸腺は無菌動物と自然動物で発育に差がない。ただ無菌動物において、リンパ節におけるT系統、胸腺依存のリンパ球密度が少い。無菌ラットでは、老令になるまで、胸腺組織内にplasma細胞が出現しないが、自然ラットでは50日頃より後に、胸腺中にplasma細胞が出現して来るようになる。

Homeostasis of cell-mediated immunity

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Homeostatic mechanisms in humoral immunity are becoming to some extent understood. For example, the production of a specific humoral antibody may be limited by a feedback mechanism involving the antibody itself, and an overall feedback may operate to control the levels of particular classes of immunoglobulins. Very little is known of the ways in which the development or expression of cell-mediated immunity (CMI) is controlled. It is clear, however, that the lymphoid proliferation in the development of a specific response does not continue indefinitely at a high level; nor does that induced by mitogens *in vitro*. Likewise, classical delayed skin reactions, while sometimes violent, appear to be self-limiting, as do other *in vivo* and *in vitro* correlates of delayed-type hypersensitivity, such as macrophage reactions and the production of lymphokines. It is the purpose of this paper to examine some possible mechanisms of homeostasis of CMI.

Our current experimental approach to this problem had its origin in studies of phytohaemagglutinin (PHA) induced lymphocyte transformation in lepromatous leprosy, a disease in which there may be a general depression of CMI. We found that, in general, the capacity of lymphocytes to transform when cultured in normal serum was not depressed. However, serum from Malay and Indian patients depressed the transformation of both autologous and normal lymphocytes, while serum from lepromatous Chinese depressed the transformation of autologous lymphocytes. Similar findings have been reported by others with respect to antigen and/or PHA induced transformation in other diseases, e.g., secondary syphilis, Hodgkin's disease, idiopathic steatorrhoea and cirrhosis. The hypothesis has been formed that the factor(s) in such pathological sera represent, not new disease-specific factor(s) but an excessive production of factor(s) which normally act to regulate CMI. Among the candidates for this role are: (a) ribonucleases; (b) "immunoregulatory alpha-globulin"; (c) other, as yet undefined, substances. The cellular source of homeostatic factor(s) may be lymphocytes themselves, macrophages or other cells. CMI is under the general control of the thymus, but this appears to have an "all-or-none" role rather than a more finely homeostatic function. Humoral antibodies may interfere with the development or expression of CMI to specific antigens. Finally, thymus-derived lymphocytes themselves may possess some inbuilt limitation to their proliferative capacity, analogous to but perhaps more marked than the Hayflick limit on other cells. Some experimental approaches to these questions will be discussed.

感作リンパ球の抗体産生における役割

野本亀久雄（九大・医・細菌）

The role of sensitized lymphocytes in the antibody production against the same antigens.

KIKUO NOMOTO(Department of Bacteriology, School of Medicine Kyushu University)

ABSTRACT: C57BL mice were grafted subcutaneously with hamster lymphoma and were injected intravenously with hamster RBC at various times after the lymphoma-grafting, and the antibody production in the lymphoma grafting mice were compared with that in the non-grafted mice. a) The lymphoma-grafted mice showed strong cytotoxic activity against hamster lymphoma or embryonal cultured cells in regional lymph node cells, but hemolysin-forming cell against hamster RBC was not detected in spleen or lymph node and gamma-globulin-producing cells were not increased in these mice. b) When hamster RBC were injected intravenously into these lymphoma-grafted mice 4 to 7 days after lymphoma-grafting, the increases in the numbers of hemolysin-forming cells and also in the diameter of hemolysin-plaques were observed 4 days after RBC injection. The enhancing capacity to produce hemolysin was transferred with sensitized regional lymph node cells into non-grafted mice. c)IgG antibody against hamster RBC was produced in the lymphoma-grafted mice 10 to 20 times as much as in non-grafted mice 7 days after RBC injection. d) The time course of conversion from IgM to IgG production took the same course as the primary response against sheep RBC.

Ⅰ. 実験目的：近年生物学的実験技術の改良にともなって，細胞性免疫の反応最終過程は除々に明らかにされつつある（抗原特異的標的細胞破壊，遅延型皮内反応など）。しかし 細胞性免疫の成立成立から最終反応に至る各段階については不明といわざるを得ない。細胞性免疫の本態を明らかにするためには 1)感作リンパ球の安定化と定量化．2)感作リンパ球の持つ抗原特異的結合部位の本態．3)同一抗原に対する細胞性免疫と抗体産生の相互関係などの点の解決が必要であろう。本論文では，感作リンパ球の抗原特異的結合部位の解析の第一歩として，細胞性免疫に感作された動物のリンパ球が，同一抗原に対する抗体産生においてどのように働くかを研究目的とした。

Ⅱ. 実験材料：同一抗原決定基にたいし，細胞性免疫と抗体産生とは，より分離独立して発生する実験系が本研究には必要である。異種のくみ合せで，赤血球を抗体産生の免疫原とし，腫瘍細胞と正常細胞（リンパ節細胞，胸腺細胞，脾細胞など）を細胞性免疫の免疫原として多くの系を得た。すなわち，ハムスター体細胞（リンホーマ，胸腺細胞，リンパ節細胞）とハムスター赤血球がもっとも適した系であることを見た（recipientはマウス）。

ハムスター休細胞は免疫原としての赤血球抗原はほとんど認められないが、赤血球が前もってほかの凝集素又は沈血素と吸收するオ(反応原としての赤血球抗原)は、赤血球の方へ多である。

II. 実験成績:

1) ハムスターリンパーマを皮下移植されたマウスの免疫反応。(a) リンパーマ 1×10^7 を側腹部両側皮下に移植すると、4～8日目にいちがるしい所所リンパ節の肥大と所所リンパ節細胞にハムスター細胞による細胞障害像が認められる。脾も正常の2～4倍に腫大する。(b) リンパーマ移植後4～8日にリンパ節および脾にハムスター赤血球による抗体産生細胞(PFC)及びマクロファージロゼリン産生細胞(蛍光抗体の増加)はみとめられなかった。また血清中には抗赤血球、抗リンパーマ、抗リンパ球抗体は認められなかった。(c) リンパーマ移植マウスの腹腔浸出細胞と赤血球とを用いて migration inhibition test を行うと、軽度の遊走抑制がみとめられた。

2) 沈血素産生細胞(plaque-forming cell)。(a) リンパーマ移植4～7日目に、ハムスター赤血球を静注し、さらに4日後に脾およびリンパ節のPFCを観察した。非移植群にハムスター赤血球を静注した場合、脾あたり2,000～8000のPFCがみとめられるのみであるが、リンパーマ移植群に赤血球を静注すると24,000～90,000のPFCがみとめられた。このPFCの増加はハムスター赤血球に対する抗原特異的現象であった。(b) リンパーマ移植群のPFCの直径は非移植群にくらべて2.0～2.5倍であった。(c) リンパーマ移植－赤血球静注群がリンパ節細胞にはPFCはみとめられなかった。(d) PFC産生の促進効果はリンパーマ移植群の所所リンパ節細胞で passive transfer される。(e) リンパーマ移植後9日以降に赤血球を静注すると、促進効果は殆ど低くなる。(f) ハムスター赤血球をいろいろの濃度で皮下注射し、6～7日後赤血球を静注しても、PFCの産生促進はみられない。(g) 赤血球を静注し、6-7日後再び赤血球を静注しても、PFCの産生促進はみられない。

すなわち、リンパーマ移植によって、赤血球抗原に対しては免疫反応の準備状態(抗原認識細胞の出現で中断)まで進められたと考えられる。抗体産生の典型的二次反応ということとなり、リンパーマ移植－赤血球静注群では5日以内はIgG抗体はみられない。

3) 凝集素(IgGとちがう)の産生:(a) 非移植マウスにハムスター赤血球を静注しても、7日以後の抗体価は低く、約半のマウスでは凝集素は検出されず、残りの者で2-4倍みとめられることがまい。一方、リンパーマ移植群に赤血球を静注すると、全例に32～128倍の凝集素がみとめられる。(b) 非移植－赤血球静注群では7日以後も2ME resistant な凝集素は検出されず

なお、リンパ球移植群では全例に検出される。(i) IgM→IgG産生への転換は、リンパ球移植－赤血球静注群で、ニワトリ赤血球に対する一次反応と同じ時間的経過と抗体価の変動を示す（静注3～5日 IgM、7日以後IgG）。(d) ハムスター赤血球を静注し、6～7日後赤血球を再び静注すると、3～4日にすでにIgG抗体に入れかわる。

すなわち、リンパ球移植－赤血球静注群では、IgG産生は量的に増強されるが、そのパターンは強い抗原（ニワトリ赤血球）に対する一次反応の時間的、質的経過を示す。この点からも、リンパ球移植によって、免疫反応が抗原認識細胞の出現で中断していないと考えられよう。

4) ハムスター胸腺細胞（リンパ球の代り）と赤血球を同時に静注すると、所ぉょびIgG産生はいちじるしく抑制される。(i) しかし、胸腺細胞のみまたは赤血球とともに静注したマウスの血清中に、抗胸腺細胞抗体も検出されない（7日以内）。(b) これらの動物に6～7日後赤血球を静注すると、抗体産生の促進がみとめられる。このことは、胸腺細胞と赤血球の結合によって、同一抗原に対応する免疫適格細胞が、細胞性免疫に進んだことを示唆する。

5) ニワトリ赤血球（強い抗原）とハムスター赤血球（弱い抗原）の抗原競合：非移植マウスにニワトリ赤血球とハムスター赤血球を同時に静注すると、ハムスター赤血球に対する抗体とくにIgG抗体の産生が抑制される。一方、リンパ球移植群ではニワトリとハムスターの赤血球を同時に静注しても、ハムスター赤血球に対する抗体産生は抑制されない。すなわち、リンパ球を移植することで、元来弱い抗原であるハムスター赤血球に対する認識機構が増強され、強い抗原（ニワトリ赤血球）によって抑圧されないことが示唆された。

IV. 結論

リンパ球が胸腺細胞が赤血球抗原を反応原として所有していることから、上記の成績を次のように解釈し、今後の研究の方向としている。リンパ球は相識抗原に対する細胞性免疫を成立させると同時に、赤血球抗原においても速やかに感作する。この状態では、赤血球抗原と認識する活性の高い抗原認識細胞（感作リンパ球）が存在する。抗体産生に動員のよい形で赤血球抗原が入ると（赤血球静注）、これらの細胞が抗体産生に関与する。しかし、リンパ球移植時には抗体産生までは進んでいないので、免疫反応の強さは増大するが、抗体産生のパターンは一次反応の時間的経過をとる。このような考えを基盤にし、リンパ球移植マウスで抗体産生の促進に働く細胞が感作リンパ球か抗体産生に個有のものかを解析中である。

白血球抗原の免疫系統発生学的研究

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ABSTRACT:

There has been no report of phylogeny of leucocyte (lymphocyte) antigen. We have investigated phylogeny of HL-A antigen which plays a major role of transplantation antigen or histocompatibility antigen. Animals used were lamprey, tortoise, hen, rabbit, rat, mouse, cow and pig. Lymphocyte, occasionally leucocyte were collected from the peripheral blood in each animal. The lymphocyte (leucocyte) antigen was tested by modified method of Dr. Amos, micro-lymphocytotoxicity with identified HL-A antisera 1, 2, 3, 5, 7, 8, 9, etc. sent from NIH. HL-A antisera were absorbed with each animal's red blood cell previously. Both absorbed and non-absorbed antisera were used.

Results were shown in Figs 1 - 9. There were discovered HL-A ? 1, 2, 8, 9 in lamprey, no HL-A antigen in tortoise, probably negative and ? 2, 8, HL-A 1, 3, 7, 8, 9, ? in rabbit, HL-A ? 1, 2, 3, 5, 7, 8 in rat, HL-A 1, 2, 3, 5, 7, 8, 9 in mouse, no good result obtained in cow and HL-A 1, 2, 3, 5, 7, 8, 9, 10, 12, 4a, 4b etc. in pig. However the aboved mentioned results were presented as a preliminary data, further study of leucocyte antigen or lymphocyte antigen will be necessary.

免疫生物学の研究が進むに従って、リンパ球の免疫機構に対する感心が深まってきた。リンパ系の系統発生的、形態学的および機能的発達段階から考察する時、最も下等な脊椎動物、円口類、ヤツメウナギにおいて、原始胸腺の出現と共に、リンパ球も出現し細胞免疫現象が認められることが知られている。最近では、移植免疫現象の立場から、その系統発生学的研究も検討されている。

ヒト臓器移植の基礎的な面から、組織適合性抗原として白血球抗原が近年注目をあび、ＨＬ－Ａ抗原としてその研究の重要性と応用価値などが検討されて来た。しかし　ＨＬ－Ａ抗原の人類学的検討は行われているが、まだその系統発生学的研究は報告されていない。この様な観点から、ＨＬ－Ａ抗原がどの種属において発見されるものか、所謂ＨＬ－Ａ抗原の系統発生樹を推定樹立することを目的とし、この結果　出来うるならば異種血清からヒトＨＬ－Ａ抗体の作成の可能性を追求しようというものである。

動物は、ヤツメウナギ、カメ、ニワトリ、ウサギ、ラット　マウス、ウシ、ブタ、ヒトを対象とした。抗血清はＮＩＨより送付されたＨＬ－Ａ抗体、ＨＬＡ 1, 2, 3, 5, 7, 8, 9 (2 - 50 - 60 - 03 - 23 - 03 Morrison, 2 - 50 - 6 - 03 - 01 - 04 Pinquette, 2 - 50 - 6 - 09 - 23 - 01 Storm, 2 - 58 - 9 -

02-03 Abruzzo C, 2-58-9-02-28-01 McCutchen M, 2-50-6-06-21-02 Shayra, 2-50-8-02-19-01 Jones) を用いた。吸収抗血清としてそれぞれの動物の赤血球により検査前に型の如く3回吸収操作を行った。補体はヒトAB型赤血球吸収家兎補体を使用した。原則として Amos法の改良 micro-lymphocytotoxicity test を行い、トリパンブルーで染色細胞の%を算定。染色細胞30%以上を陽性と考えた。白血球抗原としては各種動物から出来るだけリンパ球を純粋に採取したが、やもうえざる場合は白血球を使用した。

(リンパ球と白血球採取法); ①ヤツメウナギ白血球 — i)ヘパリン加血液採取, ii) 10% Dextran Solution in 5% Glucose を加え4~5時間赤血球を沈降させる, iii)下戸に赤血球が沈降し、赤血球の最上戸に白血球戸が出来るので、小パイペットで白血球のみを採取する, iv)円口類生理的塩類* に浮遊使用する。* Nacl 0.81%, Kcl 0.021%, $CaCl_2$ 0.023%
②カメ白血球 — i)ヘパリン加血液、室温で2~3時間放置, ii)下戸に赤血球、上戸に白血球が浮遊する。iii) 800 rpm 8分遠沈, iv)上清をすてる。v)沈渣に爬虫類生理的塩類* を加え白血球浮遊液を作成する。* Nacl 0.81%, Kcl 0.022%, $CaCl_2$ 0.026% ③ニワトリリンパ球 — i)ヘパリン加血液採取, ii)そのまゝ 800 rpm 8分遠沈, iii)下戸に赤血球上戸に白血球浮遊液が出来る, iv)注意深く赤血球の混入のない様に上清をとる, v) 800 rpm 8分遠沈、上清をすて沈渣に barbitol buffer 3~5 ml を加える。vi)ヒト用 Δ10 fiber column に通す vii) 90~96% viable, 4×10^6/ml リンパ球を採取出来る。 ④ウサギリンパ球 — i)ヘパリン加血液採取。ii)プラスマジェルを加える iii)上戸の白血球戸をとり 800~1000 rpm 8分遠沈、その上清をすてる。iv)沈渣に barbitol buffer を加える v) Δ10 fiber column に通す vi) 800~1000 rpm 8分遠沈 vii)沈渣を barbitol buffer に浮遊させる ⑤ラットおよびマウスリンパ球 — i)マウスは眼窩静脈叢より、ラットは断時 静脈血を採取 ヘパリンを加える。ii)プラスマジェルを加える マウスは5~6 × 0.6~0.8 cm の試験管を使用。iii)上戸の白血球戸を採取 iv) 800 rpm 8分遠沈、上清をすてる v)沈渣に barbitol buffer を加える。vi)我々の考案したマウス用 Δ10 fiber column (小カラム) およびラット用 Δ10 fiber column に通す vii) barbitol buffer に再浮遊 viii) 800~1000 rpm 8分遠沈 ix)沈渣に Tris-NH_4Cl 3 ml を加え溶血させる 一回の操作がのぞましい。x)遠沈右 barbitol buffer に浮遊使用する。 ⑥ブタリンパ球 — ヒト末梢血リンパ球採取法に順ずる*。リンパ球は98~99% viable, $5 \sim 6 \times 10^7 \sim 10^8$/ml 採取可能。* 第4回免疫生物研究会 免疫実習参照。

使用した各種動物、ヤツメウナギ、カメ、ニワトリ、ウサギ、ラット、マウス、ウシ、ブタのリンパ球又は白血球の抗原性を、ヒトHL-A抗体により検査した結果を、細胞の染色性(%)で表現し表1～9に示した。

1)ヤツメウナギ白血球　表1；北海道より春と秋に空輸されるものを使用したので、吸収血清群のテストは現在のところまだ行っておらない。非吸収群でもなお80%の強陽性のものを一応HL-A抗原陽性と考えた。検査された5匹ではHL-A1, 2, 8が1/5匹に、HL-A9は3/5匹に証明された。今后検査数をまし、吸収血清について検討しなければならない。　2)カメ白血球　表2；検査された7匹中、全例にHL-A抗原は陰性であった。従ってカメ白血球にはHL-Aは存在しないか、現在の方法論では証明出来ないと考えた。　3)ニワトリリンパ球　表3；表3に示す如く検査数は5羽の少数例ではあるが、リンパ球(3羽)では吸収群の方が非吸収群より陽性度が強くなることは、検査法に何んらかの不備があるものと考える。従って非吸収群について検討すると、80%以上の強陽性はなく、50%以上のものはHL-A 2, 8がひっかかった。残り2羽の白血球では、HL-A 8で50%陽性を示した。なお一層の検討が必要であるが、HL-A抗原は非常に弱いものが存在しないものと考えられよう。　4)ウサギリンパ球　表4；8羽のウサギリンパ球抗原の結果は表4に示した如くで、血清吸収群において明らかな陽性を示すものを認めた。しかし個体差があり、全くHL-A抗原を欠如するものも存在した。

5)ラットリンパ球　表5；ラット全体としては12匹、そのうちSprague-Dawley, Donryu, Long-Evansを使用したが、全体的にその反応性は弱く、ラットリンパ球抗原のHL-Aは1, 2, 3, 5, 7, 8で弱陽性又は疑陽性と考えられよう。　6)マウスリンパ球　表6；マウス8匹うちBALB/c, ddOS, DBA/2, C57BLの各2匹について検討した結果、非吸収群で強陽性、吸収群でなお陽性を示すものを認め、HL-A 1, 2, 3, 5, 7, 8, 9すべて存在することを示唆している。　7)ウシ白血球　表7；ウシ白血球の分離は、赤血球の混在がかなりあり、検査された6匹に関しては殆んど陰性であったが再検が必要である。しかしこの白血球浮遊液には、かなりの顆粒球が存在しているのに、なお陰性傾向を示したことは、今后にいろんな考察材料を残した様である。　8)ブタリンパ球　表8；ブタ10頭のリンパ球を検討すると表8の如く、HL-A 1, 2, 3, 5, 7, 8, 9, 10, 12, 46、その他が陽性に証明された。ブタ個体差がありHL-A陰性のものもあるが、HL-A陽性度により2-3群に分類可能であろう。

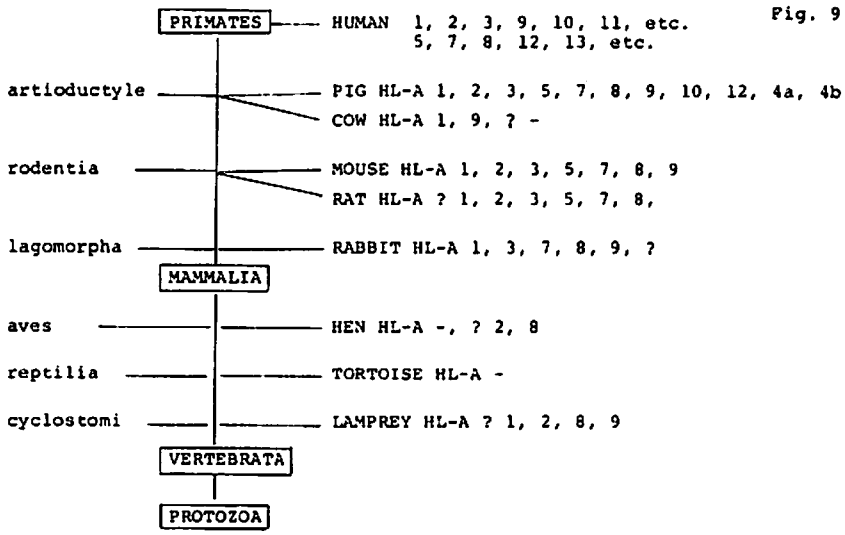
ヒト動物の白血球、リンパ球抗原の系統発生学的研究は、世界中まだそ

の報告をみない。従ってその検査法、データーの解析など不完全な部分も多いが　一まずpreliminary dataとして報告した。

検査した各種動物のリンパ球（白血球）抗原のＨＬ－Ａ抗原陽性率をまとめ、これを動物系統発生樹に添加してみると表９の如くになる。はたしてＨＬ－Ａ抗原は動物白血球抗原において、その系統発生段階では、どの階段で発生したものか結論づけるには、なお多くの問題があり、ここでは話題を提起し諸先輩の御批判をあおぐ次第である。

EVOLUTIONAL TREE OF ANIMALS & PROSPECTIVE PHYLOGENY OF HL-A ANTIGEN

Fig. 9



HL-A ANTIGEN AND LAMPREY LEUCOCYTE ANTIGEN

Fig. 1

NIH LEUCOCYTE ANTISERA	HL-A	L1	L2	L3	L4	L5
2-50-6-03-23-03 Morrison	1	90	50	10	50	0
2-50-6-03-01-04 Pirquette	2	50	10	30	90	70
2-50-6-09-23-01 Storm	3	30	0	40	10	60
2-58-9-02-20-03 AbruzzoC	5	30	10	50	0	30
2-58-9-02-28-07 McCutchenM	7	10	0	0	20	70
2-50-6-06-21-02 Shayra	8	50	0	40	90	50
2-50-8-02-19-01 Jones	9	90	80	90	60	60

Absorved antisera not tested

HL-A ANTIGEN AND TORTOISE LEUCOCYTE ANTIGEN

Fig. 2

NIH LEUCOCYTE ANTISERA	HL-A	T1	T2	T3	T4	T5	T6	T7
2-50-6-03-23-03 Morrison	1	0 (0)	0(0)	0(0)	0(10)	0(30)	0 (0)	0(10)
2-50-6-03-01-04 Pirquette	2	10(10)	10(0)	0(0)	0(10)	0 0	10 (0)	0(10)
2-50-6-09-23-01 Storm	3	0 (0)	0(0)	0(0)	0(10)	0 10	20(10)	0(10)
2-58-9-02-20-03 Abruzzo C	5	0 (0)	0(0)	0(0)	0(20)	0 0	0(10)	0 0
2-58-9-02-28-07 McCutchenM	7	0 (0)	0(0)	0(0)	0 ?	0 0	0(10)	0 0
2-50-6-06-21-02 Shayra	8	0 (0)	?(0)	0(0)	10 10	0 0	10 (0)	0 0
2-50-8-02-19-01 Jones	9	0 (0)	80(0)	70(0)	10 ?	0 0		

( ): Absorbed antisera

HL-A ANTIGEN AND HEN LYMPHOCYTE ANTIGEN Fig. 3

NIH LEUCOCYTE ANTISERA	HL-A	H1	H2	H3	H4	H5
2-50-6-03-23-03 Morrison	1	20(50)	20(50)	30(50)	20	25
2-50-6-03-01-04 Pirquette	2	50(80)	45(60)	50(50)	30	20
2-50-6-09-23-01 Storm	3	30(55)	30(50)	35(50)	15	40
2-58-9-02-20-03 Abruzzo C	5	30(75)	30(70)	20(20)	25	5
2-58-9-02-28-07 McCutchenM	7	40(45)	45(50)	40(40)	40	30
2-50-6-06-21-02 Shayra	8	30(50)	30(30)	60(60)	50	20
2-50-8-02-19-01 Jones	9					

( ): Absorbed antisera

H1-3: Lymphocyte, H4,5: Leucocyte

HL-A ANTIGEN AND RABBIT LYMPHOCYTE ANTIGEN Fig. 4

NIH LEUCOCYTE ANTISERA	HL-A	R1	R2	R3	R4	R5	R6	R7	R8
2-50-6-03-23-03 Morrison	1	50 (0)	50 (0)	50(50)	10 (0)	40(10)	?(10)	100(10)	50(30)
2-50-6-03-01-04 Pirquette	2	10 (0)	10 (0)	40 (0)	30 (0)	30 (0)	50(10)	100(20)	80(20)
2-50-6-09-23-01 Storm	3	10 (0)	50 (0)	50(10)	10 (0)	20 (0)	?(40)	60(10)	100(30)
2-58-9-02-20-03 Abruzzo C	5	0 (0)	0 (0)	10(10)	0 (0)	30 (0)	60(20)	80(20)	100(20)
2-58-9-02-28-07 McCutchenM	7	30(20)	0 (0)	0 (0)	0 (0)	50(20)	40(30)	100(30)	100(10)
2-50-6-06-21-02 Shayra	8	40 (0)	30(20)	50(30)	20 (0)	40(30)	80(40)	100(10)	80(10)
2-50-8-02-19-01 Jones	9	50(10)	40 (0)	50(40)	0 (0)	60(10)	40(10)	80(40)	100(20)

( ): Absorbed antisera

HL-A ANTIGEN AND RAT LYMPHOCYTE ANTIGEN

Fig. 5

NIH LEUCOCYTE ANTISERA	HL-A	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12
2-50-6-03-23-03 Morrison	1	80(20)	40(30)	10(10)	60 (5)	70 (5)	60(70)	70(10)	50(40)	50(30)	50 (0)	50(25)	70(10)
2-50-6-03-01-04 Pirquette	2	80(10)	30(40)	30(10)	80 (0)	30(10)	80(30)	60(50)	100(30)	60(40)	35 (5)	50(40)	60 (5)
2-50-6-09-23-01 Storm	3	50(20)	50(30)	10(20)	20(10)	20(20)	70(40)	10(35)	80(50)	70(20)	10 (0)	30(10)	30 (0)
2-58-9-02-20-03 Abruzzo C	5	30(30)	40 (0)	10(10)	5 (5)	30 (5)	30(20)	10(40)	70(50)	80(30)	20 (5)	20(20)	10 (0)
2-58-9-02-28-07 McCutchenM	7	80(30)	30(50)	20(10)	90 (5)	40(10)	30 (0)	30(10)	90(60)	60(40)	10(10)	20(10)	40(10)
2-50-6-06-21-02 Shayra	8	50(20)	40(50)	20 (0)	40 (5)	50 (5)	40 (0)	20 (0)	80(40)	80(30)	20(10)	40(10)	50 (0)
2-50-8-02-19-01 Jones	9	80(10)	30(30)	20 (0)	50 (5)	40 (5)	50 (5)	80(10)	60(70)	60(20)	50 (0)	30(30)	40 (0)

( ): Absorbed antisera

R1-5: Sprague-Dawley, R6, 7: Donryu, R8-12: Long-Evans

HL-A ANTIGEN AND PIG LYMPHOCYTE ANTIGEN

Fig. 8

NIH LEUCOCYTE ANTISERA	HL-A	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
2-50-6-03-23-03 Morrison	1	60 (0)	80(40)	60(50)	80(50)	50 (?)	50(40)	50(30)	60(20)	40(20)	60(20)
2-50-6-03-01-04 Pinquette	2	100 (0)	100(50)	60(50)	50(50)	100 (?)	50(10)	50(40)	60(20)	50(10)	80(10)
2-50-6-09-23-01 Storm	3	100 (?)	50 (?)	80(...)	60(60)	80 (?)	70(50)	40(50)	50 (?)	40 (?)	20(10)
2-58-9-02-20-03 Abruzzo C	5	100(10)	50(40)	50(60)	80(20)	100 (?)	80(10)	50(40)	40(40)	30(20)	60(10)
2-58-9-02-28-07 McCutchenM	7	100(40)	80(50)	80(50)	80(50)	80 (?)	60(40)	50(30)	60(20)	50(20)	50(10)
2-50-6-06-21-02 Shayra	8	80(60)	50(80)	100(50)	40(50)	100 (?)	50(50)	100(40)	100(40)	100(40)	100(10)
1-01-9-07-17-03 Petrault	9	100(70)	80(60)	100(90)	80(40)	80 (?)	40(30)	100(40)	100(10)	80(40)	100(10)
2-61-9-04-10-01 Beegle	10	80(50)	60(30)	60(50)	60(30)	60 (?)	40(20)	50(20)	60(20)	90(40)	100(50)
1-07-8-01-18-01 Daines	12	100(80)	50(40)	80(70)	80(30)	50 (?)	60(10)	100(20)	100(10)	90(40)	100(40)
1-01-8-12-22-01 Chamard P	4a	50(20)	50(40)	50(50)	20(40)	lis (?)	50(10)	50(10)	20(10)	30(20)	50(10)
1-04-9-07-12-01 12vanTongeren,	4b	100(50)	80(50)	60(50)	90(40)	100 (?)	50(40)	100(10)	100(20)	100(30)	100(20)
2-55-8-12-10-01 Lee C	?	40(50)	80(60)	50(50)	100(20)	lis(10)	lis(40)	50(40)	lis(10)	0 28	80(40)
2-50-6-04-12-01 Siah	?	50 (0)	50 (0)	80 (0)	40 (?)	40 (?)	70(20)	50(60)	10(10)	20(20)	50(10)
2-50-6-04-21-01 Thompson	non	80(50)	80(60)	80(60)	80(50)	100 (?)	80(40)	50(40)	40(10)	20(10)	10(10)

( ): Absorbed antisera

HL-A ANTIGEN AND MOUSE LYMPHOCYTE ANTIGEN

Fig. 6

NIH LEUCOCYTE ANTISERA	HL-A	M1	M2	M3	M4	M5	M6	M7	M8
2-50-6-03-23-03 Morrison	1	80(50)	50(40)	50(40)	80(80)	100 (0)	50(30)	100(10)	100(50)
2-50-6-03-01-04 Pirquette	2	100(30)	60(30)	100(30)	80(40)	80(20)	100(20)	100(40)	100(50)
2-50-6-09-23-01 Storm	3	80(10)	60(20)	80(40)	80(50)	100(30)	80(30)	100(30)	100(60)
2-58-9-02-20-03 Abruzzo C	5	100(30)	50(30)	50(50)	100(30)	100(50)	50(20)	100(50)	50(50)
2-58-9-02-28-07 McCutchenM	7	60(50)	50(40)	100(20)	70(50)	80(80)	50(40)	100(40)	100(20)
2-50-6-06-21-02 Shayra	8	80(80)	100(40)	80(40)	80(60)	100 (?)	100(30)	100(100)	100(40)
2-50-8-02-19-01 Jones	9	100 (?)	80 ?	90(50)	80(50)	100 (?)	100(50)	100 (?)	100(40)

( ): Absorbed antisera

HL-A ANTIGEN AND COW LEUCOCYTE ANTITEN

Fig. 7

NIH LEUCOCYTE ANTISERA	HL-A	C1	C2	C3	C4	C5	C6
2-50-6-03-23-03 Morrison	1	30(10)	20(10)	30(40)	80(40)	80(10)	30(20)
2-50-6-03-01-04 Pirquette	2	20(20)	10(10)	20(40)	80(20)	80(10)	40(10)
2-50-6-09-23-01 Storm	3	40(10)	10(10)	10(50)	40(20)	40(10)	10(20)
2-58-9-02-20-03 Abruzzo C	5	10 (0)	5(20)	20(40)	20(10)	10(10)	10(10)
2-58-9-02-28-07 McCutchenM	7	10 (0)	10(10)	20(20)	10(10)	20(10)	10(10)
2-50-6-06-21-02 Shayra	8	10(10)	10(10)	10(30)	10(10)	0(10)	20(20)
2-50-8-02-19-01 Jones	9	40(20)	50(40)	10(40)	10 ?	20(20)	10(20)

( ): Absorbed antisera

SURFACE IMMUNOGLOBULIN--MOIETIES ON LYMPHOID CELLS

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Viable cells isolated from several Burkitt lymphomas and other lymphoid malignancies were shown to react with fluorescein labelled anti-immunoglobulin sera. The evidence that most of the immunoglobulin is localized on the cell surface is provided by the fluorescence staining pattern of fixed cells, as only the periphery of cells reacted. In all the cases encountered the cells reacted with antibodies specific for the heavy chain of IgM (μ) and χ light polypeptide chain. In the presence of complement, these antibodies were cytotoxic. Cells from such Burkitt lymphomas which have been kept in culture for more than a year have maintained the μ and χ structures, proving that these are synthesized by the cells. The ultrastructure of the cells was basically similar to that of lymphoblasts.

When a number of lymphoid malignancies were surveyed it was observed that the strength of reactivity with anti-immunoglobulin sera varied among different patients. As these cells may be regarded as a malignant clonal outgrowth of immunoglobulin carrying normal lymphocytes maintaining their product of differentiation, they provide a good tool for studies on the properties of the immunoglobulin moiety itself and its relation to the cell membrane.

An attempt was made to estimate the amount of cell membrane bound immunoglobulin-structures with special reference to two cell populations, T.P. chronic lymphocytic leukaemia cells obtained directly from the patient and a culture line Daudi established from a Burkitt lymphoma. The methods used were:

1. Direct quantitation of the bound fluorescein-labelled antibody.
2. Quantitative absorption of antisera, and assay of residual activity, by passive haemagglutination, by cytolysis or by estimation of the staining intensity of IgM carrying cells.

It was shown that 10^9 viable or frozen T.P. and Daudi cells seem to have an amount of μ moieties equivalent to the μ chains of approximately 25 ug IgM.

Studies on the T.P. cells indicated that the immunoglobulin structures were liberated from the surface membranes by homogenization. The size of these structures was determined by sucrose density gradient ultracentrifugation and gel filtration on Sephadex G-200, and indicated that the immunoglobulin is present as 7-S subunits. Accordingly the approximate number of molecules per cell is about 80,000. Estimation of the distances between the molecules is probably less meaningfull as immunofluorescence showed that they are unevenly distributed on the cell surface.

Fluorescein conjugated anti-immunoglobulin antibodies were also found to bind to the surface of a fraction of viable rabbit, mouse and human cells from various organs. The types of cells have as yet not been determined but their appearance are compatible with lymphoid cells.

If the fact that only μ and χ specificities were demonstrated by the immunofluorescence on the cells derived from lymphoid malignancies reflects properties of normal lymphoid cells it may indicate that cells with other immunoglobulin classes if present, have a lower concentration of the immunoglobulin.

From the information available on the relationship of the immunoglobulin classes to antigens this fact might be explained in the following way: It is known that large molecules with many identical antigenic determinats provoke the production of IgM-antibodies more easily than small molecules. The converse is to some extent true for the production of IgG-antibodies. Moreover, IgG-molecules have been shown to have a higher affinity for antigenic determinants than IgM-molecules. IgM-molecules have ten, IgG-molecules have two combining sites. One may consider cells with immunoglobulin-structures on their surface as giant antibodies. For the binding of antigens to the cell, the concentration and density of combining sites, and their affinity for the antigen are important factors. In order to explain how cells producing IgM compete efficiently with IgG-producing cells for large antigen molecules, it has been assumed that the IgG- and IgM-receptors resemble IgG- and IgM-molecules in serum. The distance between several IgM-combining sites should then be comparatively smaller than the distance between a similar number of IgG-combining sites.

By visual observations it was noticed that the intensity of staining of the Daudi culture cells by the conjugated anti IgM serum varied somewhat on different occasions. The amount of IgM was therefore quantitated daily after seeding a culture at 10^5/ml density.

The results are given in Fig. 1 and demonstrate that during the growth period fluctuations were found to occur, which is reflected also in the sensitivity of the cells to the cytotoxic effect of anti IgM serum.

Hybrid cells between the IgM positive Daudi cells with a mouse fibroblast (L cell) did not maintain the production of the immunoglobulin. The hybrid character of the cells was checked by the presence of human and mouse chromosomes and the isoantigens derived from both species.

Friend virus感染ラットのラント症候群

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Runting syndrome of the rat infected with Friend virus. NORITOSHI TAKEICHI., NOBORU KUZUMAKI and HIROSHI KOBAYASHI. (Cancer Institute, Hokkaido University School of Medicine, Sapporo)

ABSTRACT : We have noticed the occurrence of the runting syndrome of the rat during the 4 - 5th weeks after the Friend virus injection at birth. In this paper, we will described the experiments regarding the mechanism of the runting syndrome. Incidence of the runting syndrome depends on the amount of the Friend virus inoculated. Although the incidence of the runting syndrome in the rats injected with a large amount of the Friend virus($10^{4.0}$- $10^{5.0}MID_{50}$/Rat) is approximately 10 per cent, the incidence of the runting syndrome in the rats injected with a small amount of the Friend virus($10^{1.0}MID_{50}$/Rat) is approximately 70 %, which is the highest percentage among various experimental groups (Table 1). Incidence of the runting syndrome is also influenced by the time of the Friend virus inoculation. A high incidence of the runting syndrome was also observed in the rats injected with a large amount of the Friend virus, if the virus was injected at 4 - 5 days after birth. Symptoms in these runting rats are a loss of the body weight, diarrhea, ruffled hair, anemia and a wasting of the body at the late stage resulting in death. The characteristic pathological findings of the runted animals are an atrophy of the thymus and an enlargement of the spleen. Prominent destruction and disappearance of small lymphocytes was observed in most parts of the thymus(Fig. 2). There are many cases having lymph-follicles replaced by the proliferating reticulum cells in the spleen.

The mechanism of the runting syndrome seems to be related to some immunological phenomena, because the development of the runting syndrome is inhibited by X-irradiation(Table 2). Immunofluorescence studies indicate that the Friend virus antigen and Friend virus-specific membrane antigen are the highest at the 20th day after the inoculation which was followed by a lowering of the incidence at a later stage(Text-Fig. 1). Cytotoxic test indicates that the Friend virus-specific cytotoxic antibody is the highest at the 21st day after the inoculation(Text-Fig. 2). From the above results, the development of the runting syndrome in the rats injected with a certain amount of the Friend virus at a certain number of days after birth may be account for by the immunological reaction of the normal immune competent cells not infected with Friend virus, with the immune competent cells acquiring Friend virus-specific membrane antigen on the cell surface, which may be recognized as foreign by the host.

我々はマウスの白血病ウイルスとして知られているFriend virusを新生児ラットに接種すると、接種後4－5週目にかけてラント症候群の発生するもののあることに気付いた。今回これらラント症候群発生ラットを免疫病理学的に検索し、病変の発生条件および機序に関して検討した。

材料と方法

Friend virusおよびその感染方法－Friend virusはDHS/MK系マウスのFriend病脾を用いて維持継代されており、通常Chenaille法にて回収した。回収されたウイルスの原液（$10^{5.0}$- $10^{6.0}MID_{50}$/Rat）或いは階段稀釈した液0.3 mlを新生児ラットの皮下および腹腔内に接種した。

動物－　近交系　Wistar King Aptekman/MK　ラットの雄、雌を使用した。

螢光抗体試験－　全て間接法を用いた。抗血清としては抗Friend Virus家兎血清を用い、細胞はFriend virus接種ラットの胸腺、脾より細胞浮遊液を作成しaceton固定および非固定材料にて検索した。

細胞障害試験－　Trypan blueによるDye exclusion methodを用いた。抗血清は新生児期にFriend virusを接種されたラットから心臓穿刺により採取した。標準的細胞としてはFriend virus誘発ラット脳腫瘍(RFT)細胞を用いた。

X線照射－　^{60}Coにて150 Rの全身照射を行った。

結果

接種量と病変の発生(Dose dependency)：ラント症候群の発生率は接種ウイルス量により大きく異なる(Table 1.)。$10^{4.0}MID_{50}$/Rat以上の大量のウイルスを生後24時間以内に接種された動物では病変の発生率は低く10%前後にすぎない。しかし接種ウイルス量を減らすに従い逆に病変の発生率は増加し、$10^{1.0}MID_{50}$/Ratの微量のウイルスを接種されたラットでは22/33(66.7%)と最も高い病変の発生率を示している。

接種時期と病変の発生(Age dependency)：病変の発生率は接種時期によっても異なり、大量($10^{4.0}MID_{50}$/Rat以上)のウイルスを生後24時間以内に接種された動物では(11.7%)と低い。しかし接種時期を遅らすことにより病変の発生率は次第に増加し、生後4－5日目に大量のウイルスを接種されたラットでは11/18 (61.1%)と最も高い病変の発生率を示している。

Table 1. Development of runting syndrome in WKA/Mk rats inoculated with Friend virus at birth within 24 hours.

Titer of Friend virus	Number of litters	Incidence of runting syndrome (%)	Mean survival days (range)
$10^{5.0}MID_{50}$/Rat	7	5/46 (10.4)	25.6 (14-38)
$10^{4.0}$	8	6/48 (12.6)	33.0 (14-62)
$10^{3.0}$	7	20/36 (55.0)	39.1 (19-54)
$10^{2.0}$	5	21/35 (60.0)	40.1 (29-66)
$10^{1.0}$	5	22/33 (66.7)	30.8 (21-59)
10^{0}	5	12/38 (31.7)	22.4 (20-25)
10^{-1}	3	6/23 (26.1)	22.3 (21-23)
10^{-2}	2	1/16 (6.3)	18.0

臨床症状：ラント症候群ラットに共通した症状は体重の増加停止、慢性の下痢、毛並の乱れ、背を丸めた不安定な歩行を呈し末期には全身虚脱状態で死亡する(Figure 1)。一方大量のウイルスを生後24時間以内に接種されたラットは正常動物と同様に順調に発育し何ら特記すべき所見は認められない。

病理学的所見：ラント症候群ラットで最も特徴的なのは胸腺の著明な萎

脳と脾臓の肥大である。光顕的には病変ラットの胸腺において、皮髄境界部から皮質にかけて小リンパ球の核濃縮、核崩壊像が多数散在し髄質においては細網細胞の増生が特徴的である(Fig.2)。脾臓ではリンパ球の減少による白色髄の萎縮が目立ち細網細胞、組織球の増生によりリンパ濾胞が置換されている例が多く。リンパ節では胚中心の外層を形成しているリンパ球が消失し、胚中心の破綻、変性像も見られる。

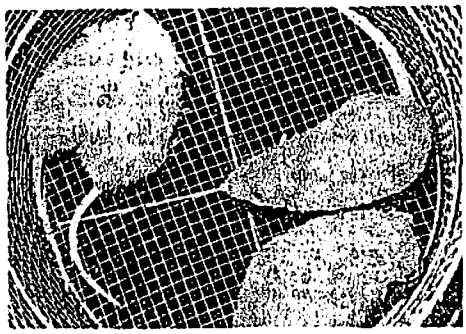


Fig. 1. Two rats in the left developed the runting syndrome after infection of Friend virus.

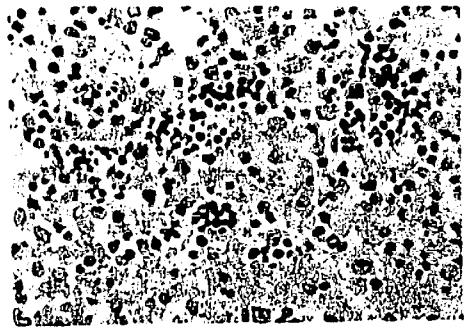


Fig. 2. Thymus from the runted rats; acute necrosis of thymic cortex is seen. H.E x 400

X線照射によるラット症候群発生の抑制：$10^{3.0}MID_{50}$/Rat のウルス量を接種されたラットの接種後7日目にX線を照射すると病変の発生率が非照射群 20/36(55.0%) に対して 4/23(17.5%) と著明に抑制された(Table 2)。

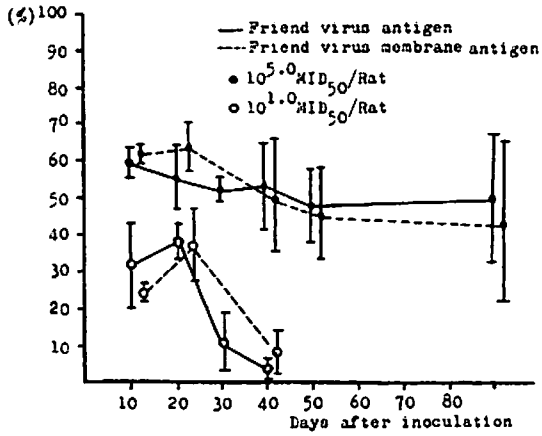
Table 2. Incidence of the runting syndrome in rats irradiated with 150 R.

Animals*	Incidece of Runting syndrome (%)	Mean survival days
X-irradiated	4/23 (17.5)	46.3
Non-treated	20/36 (55.0)	39.1

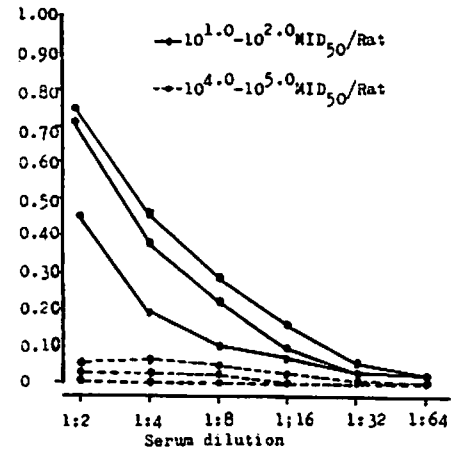
* Injected with Friend virus($10^{3.0}MID_{50}$/Rat) at birth within 24 hours.

螢光抗体法によるFriend virus抗原および膜抗原の検索：感染ラット脾細胞におけるFriend virus抗原および膜抗原を経時的に観察した結果、大量のウイルスを接種されたラットでは接種後90日以降、常に一定のFriend virus抗原および膜抗原が検索されるが、微量のウイルス接種動物では接種後20日目でウイルス抗原、膜抗原を有する細胞の数が最高となりその後急速に減少している。(Text-Fig. 1)

Friend virus特異的細胞障害性抗体の検索：微量のウイルスを接種されたラットでは接種後14日目よりFriend virus特異細胞障害性抗体が出現し、接種後21日目では検索した3例全てに最も高い抗体価が認められた。同時に行った大量のウイルス接種ラットでは全く細胞障害性抗体は検索されなかった(Text-Fig. 2)。



Text-Fig. 1. Immunofluorescence of Friend virus antigen and membrane-antigen-positive cells in the spleen from the rats inoculated with Friend virus at birth within 24 hours.



Text-Fig.2. Cytotoxic antibodies in the sera from rats inoculated with Friend virus to standard target RFT cell at the 21 day.

考察

新生児期にFriend virusを接種されたラットよりラント症候群発生が見られる。その大きな特徴は生後24時間以内に一定の微量($10^{1.0}MID_{50}$/Rat)ウイルス或いは大量($10^{4.0}MID_{50}$/Rat以上)のウイルスでも生後日数を遅らせて4－5日目に接種されたラットに最も高率に病変の発生が見られる。Friend virusによるラント症候群発生ラットでは従来の報告に見られたと同様に胸線の萎縮、脾臓の肥大が特徴的であり、光顕的にはリンパ系臓器の破壊、変性像が認められる。大量のウイルスを接種されたラットでは大部分が正常に発育し、病理学的に特記すべき所見を認めぬことと対照的である。

Friend virusによるラント症候群の発生機序については、微量のウイルス接種ラットにX線をかけると病変の発生が抑制されることから免疫反応が介在していることは明らかである。さらに螢光抗体法によるFriend virus抗原、膜抗原を検索した結果、大量のウイルス接種動物では持続的な感染状態が成立しているが微量のウイルス接種動物では、接種後20日目以後より急速にウイルス抗原、膜抗原が減少している。又細胞障害試験により Friend virusに特異的な細胞障害抗体を検索した結果、微量のウイルス接種動物の大部分が死亡するフー10日前に最も高い抗体価が証明された。

以上の点より大量のFriend virusを生後24時間以内に接種されたラットでは、その免疫担当細胞の大部分がFriend virus特異的膜抗原を獲得し"Friend免容"が成立している。しかし微量のウイルス或いは大量のウイルスでも生後日数を遅らせて接種されたラットでは膜抗原を獲得した免疫担当細胞とウイルス未感染の免疫担当細胞が一個体に共存する形となり、宿主の成熟化とともに両種免疫担当細胞間に免疫反応が発生しリンパ系臓器の荒廃をきたすものと考えられる。

ウイルス感染における細胞性免疫（アデノウイルス感染系における検討）

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Cell-mediated Immunity in Adenovirus Infection. CHUYA HAMADA, SETSUKO NAKAJIMA and HISAO UETAKE (Institute for Virus Research, Kyoto University)

ABSTRACT : The cell-mediated immunity has been suggested against virus infections from both clinical and experimental standpoints, but no adequate explanation has been proposed on its mechanism except our hypothesis, according to which on the surface of virus-infected cells there may appear antigenic change(s), which is immunologically recognized and responded as foreign by the cellular mechanism similar to transplantation immunity. This is based on the following findings: Rapid appearance of structural changes on the surface of conversion phage-infected cells, appearance of virus specific changes at the budding site of some animal virus-infected cells, and appearance of virus specific TSTA in virus-induced tumor cells.

To test our hypothesis we have examined the appearance of new antigen(s) on cells cytocidally or abortively infected with human or mouse adenovirus, and the cellular immunity induced by the antigen(s) in vivo or in vitro.

Human adenoviruses cause cytocidal or abortive infection in human or hamster cells, respectively. Virus multiplication was interrupted by the addition of FUdR. The results showed that both human and hamster cells infected with human adenovirus are capable of preventing tumor development in hamsters, which were inoculated with tumor-inducing dosis of adenovirus type 12 on the day of birth, when they were administered as immunogen on day 15 postinfection.

When C3H mouse fibroblasts were infected with mouse adenovirus at high moi, 80~90% of cells became to show viral antigens in their nuclei by immunofluorescence staining. But when the infected cells were cultured in contact with immune cells, which were harvested from spleens of mouse adenovirus immune mice, the development of the viral antigen was reduced in frequency to 1/4 of non-immune cell-treated control and the cells were killed eventually. Neither the homogenates of the immune cells nor mouse adenovirus immune sera showed similar effect. The cell damaging by the immune cells was also observed in the presence of FUdR. Protective effect of immune cells was also demonstrated in vivo by adoptive immunity test.

ウイルス感染症における防御機構として液性抗体以外に細胞性免疫の存在が諸種の知見から推察されている。特に Burton 型の agammaglobulinemia 患者がウイルス感染を正常人と同様に経過すること，新生児期，胸腺摘去動物の或る種のウイルス感染に対する抵抗力が著るしく低下することなどはその存在を強く示唆する。しかし，その成立機序については適切な説明がない。この点につきわれわれは作業仮説として，ウイルス感染により感染細胞に様々の変化（特に細胞表面の）が起り，この変化に対し細胞性免疫が整列するのではないかと考えている。この考え方は次下の諸知見に基し。ファージ感染細胞にみられる表面構造の変化，動物ウイルスの budding に際しての細胞表面膜の特異的変化，ウイルス腫瘍細胞における特異的TSTAの存在，そしてこのものに対する特異的腫瘍免疫の成立である。次下の実

験はこの作業仮説を実証するための関連実験の一部である。

実験系：アデノウイルス（Ad-V）感染系および腫瘍系を用いた。この系ではウイルス抗原とは別の特異的表位抗原またはTSTAが腫瘍および感染細胞に存在する（下記）。よって，腫瘍免疫に主役をはたすと考えられる細胞性免疫が表面変化を受けた感染細胞に対し発効することが考えられるし，また，表位抗原やTSTAがウイルス抗原とは別物であるため細胞性免疫の効果をウイルス抗原を標的とする液性抗体の作用と合わせて検討出来る可能性があることにある。

結果：①Ad-V感染細胞における特異的表位抗原の出現：アデノウイルス12型（Ad-12）トランスフォーム細胞および感染細胞にはウイルス特異的表位抗原が検出される。検出にAd-12免疫ラットの同トランスフォーム細胞培養上清免疫血清を用いた蛍光抗体法(FA)による。培養上清免疫血清はウイルス抗体を全く含まないので表位抗原はウイルス抗原とは別物と解される。事実，感染細胞における表位抗原の性状はウイルス抗原のそれとは異る。表位抗原は感染早期，ウイルス抗原に先立って検出され，FUdRはその出現を阻止しない。表位抗原はAd-12のみならず他の型のAd-V感染細胞にも認められるのでその出現はAd-V群感染細胞の普遍的属性である可能性が高い。

②：Ad-V感染細胞におけるTSTAの存在：新生児ハムスターにAd-12を接種すると約1ヶ月後から腫瘍発生をみる。この腫瘍発生に潜伏期におけるAd-12感染細胞の接種により特異的に抑えられる。この腫瘍発生抑制効果はFUdR処理感染細胞によっても得られる。感染細胞でもウイルス抗原とは別にTSTAが生産されるものと解される。

③：マウスアデノウイルス（M-Ad）感染マウスに対する免疫細胞の効果：M-AdはFL株，マウスは純系C3Hを用いた。10 LD_{50}のM-Ad腹腔内接種により感染動物は3～11日で斃死する。M-Ad免疫C3Hマウス脾臓より免疫細胞を得，M-Ad感染マウスに対する発症防御効果を調べた。免疫細胞の腹腔内接種による前処置，または同時処置でM-Ad感染マウス（10 LD_{50}, i.p.）の致命率（対照群：非免疫脾細胞処置：100%）は60～70%に低下，また斃死群においても延命効果が認められた。一方，免疫細胞破砕物は無効であった。しかし，免疫細胞集団に混在する抗体産生細胞の効果を否定には得ない。液性抗体の影響をさけるためin vitroの実験を行った。

④：M-Ad感染マウス細胞に対する免疫細胞の効果：免疫細胞は③同様に得た。M-Ad感染宿主細胞はC3Hマウス胎児細胞のcover slip培養である。ウイルス増殖の程度はFAによる核内ウイルス抗原保有細胞数(%)で示した。被検細胞にM-Adをmoi 約100で接種すると3～4日後80～90%の細胞が

核内ウイルス抗原陽性となる。これに対し，感染直前，若しくは感染後12時間以内から免疫細胞と感染細胞に接触培養（3重度：50～100）すると核内抗原の出現は約～30%の細胞に抑えられる。免疫細胞破砕物や抗ウイルス血清にはウイルス増殖抑制効果は認められない。ウイルス増殖を抑えられた感染細胞群も4日目には殆んどが死滅する。免疫細胞による細胞障害効果が考えられるがウイルスによる細胞破壊効果も否定出来ない。FUdR存在下でM-Ad感染細胞に対する免疫細胞の効果を調べた。FUdR存在下でも免疫細胞は感染細胞の約～50%に細胞障害効果を与えたが正常ラットのAd-12免疫マウス脾細胞は無効であった。

考察と結論：M-Ad感染マウス脾由来細胞はM-Ad感染細胞に特異的細胞障害効果を及ぼし，細胞内ウイルス増殖を抑える。この場合，細胞破砕物や抗ウイルス血清は無効であるので，この効果は免疫細胞自体が感染細胞に直接，接触することによる“contact effect”と考えられる。免疫細胞による同様の効果はFUdR存在下でも認められる。この条件ではウイルスやウイルス抗原の生産は認められないので，これらが免疫細胞の標的とは考え難い。Ad-12感染系からの類推として，その標的は表在抗原をもつ感染細胞そのものと推測される。

以上綜合し，アデノウイルス感染系における細胞性免疫の在り方として，ウイルス感染 ⟶ ウイルス初期蛋白の一部としての細胞表面変化 ⟶ 表面変化細胞に対する細胞性免疫の応答が考えられる。

マウスの3型レオウイルス感染症における抗リンパ球血清の影響

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Effect of Antilymphocyte Serum on Reovirus Infection of Mice.

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Reovirus infection has been known to cause an aoute, sometimes fatal disease in new-born mice, but not in older mice. Appearance and disappearance of acute symptoms in the viral infection may be greatly influenced by an age-dependent defense-mechanism of the host. This host defense-mechanism includes cellular and humoral immune-responses, production of interferon or other physiological factors.

This tige, we studied on a disease-evoking effect of antilymphocyte serum on the reovirus type 3 infection of mice and discussed the significance of cellular immune-mechanism in the viral infection.

Materials and Methods.

1) Mice: The dd strain of inbred albino mice was used.

2) Virus strain: Dearing strain (Sabin) of reovirus type 3 was propagated in L cells maintaining in YLE medium supplemented with 2% equine serum. The virus was harvested by consecutive freezing and thawing method. Then this virus fluid was centrifuged at 2,000 rpm for 15 minutes and supernates stored at -20°C until use.

3) Virus inoculation to the mice: 0.04 ml of the virus fluid (about 10^4 TCD_{50}), corresponding to about 100 LD_{50} against 2-day-old mice, was inoculated to 7 and 8-day-old mice subcutaneously.

4) Titration of serum antibody: Antibody of reovirus type 3 in sera of the mice were titrated by an indirect immunofluorescence.

5) ALS: Preparation of ALS was principally followed to the procedure described by Levey and Medawar. Rabbits were injected intravenously with approximately 10^8 lymphocytes collected from thymuses of 4-week-old dd mice 4 times every other week. The rabbits were bled one week after the last injection. The antisera were pooled and absorbed with the mouse erythrocytes until complete removal of the hemolytic activity. After the absorption, the serum was inactivated at 56°C for 30 minutes, passed through Millipore membrane and then stored at -20°C.

6) Administration of the ALS: About 0.02 ml of ALS per gram of mouse body weight was injected into peritoneal cavity 4 hours before the virus inoculation.

Results

1. Age-dependency of the manifestations of acute disease in reovirus infected mice. The morbidity and the mortality of the reovirus infected mice were first examined in connection with their ages. The clinical features of the acute disease observed in the present studies were principally similar to the description of the previous reports. The results of a typical series of experiments are summarized as follows: manifestations of the acute symptoms were evident all 2 to 5-day-old mice. The mortality was very high in the 2 and 3-day-old mice but low in the 4 and 5-day-old mice. The acute disease was observed in only a portion of 6-day-old mice inoculated with the virus and this age group also showed very low mortality. None of the acute symptoms appeared in the 7-day-old mice during 4 weeks' observation.

2. Effect of treatment with ALS on the 7 and 8-day-old mice infected with the virus. Based on the preceding findings, the effect of ALS was studied on the reovirus infection of mice. They were treated with ALS 4 hours before the virus inoculation. The morbidity and the mortality of the ALS-treated infected mice was directly compared with that of the ALS-non treated infected mice. In parallel to this, a possible toxic effect of ALS on normal mice was tested. A summary of the results is as follows: All of the mice infected but not treated with ALS did not show any sign of the acute disease. None of the toxic effect of ALS was observed on animals except being found lymphopenia. The typical acute disease due to reovirus infection was manifested in all mice treated with ALS before virus inoculation. The symptoms appeared in the mice of this group were comparable to those observed in the younger mice infected with the virus, but not treated with ALS.

3. Humoral antibody-response in the infection of mice treated with or non-treated with ALS. It was of interest to see whether the treatment of mice with ALS causes the change in humoral antibody response against the infection of reovirus.

The antibody titers of sera from mice were examined in 3 groups. Apparent healthy mice aged 6 weeks gave no detectable antibody to reovirus type 3. On the 4th week after the infection to 2-week-old mice, the high titer of antibody was observed in all of the mice examined. This results may indicate that the reovirus infection was established in these mice which did not show any acute illness. In the next place, all of the mice pretreated with ALS before the virus inoculation showed a development of the high titer of antibody. This indicates that the production of humoral antibody examined on the 4th week after the virus infection was not suppressed by this procedure using ALS before the virus inoculation.

Discussion

The present studies clearly indicated the effectiveness of ALS in the increment to host's susceptibility and in the potenciation of pathologic consequences by reovirus type 3 infection. That is, on above 7 days the mice did not manifest the acute disease by the virus infection, but it became to be apparent by pretreatment with ALS. It may be emphasized that a single injection of a small dose of ALS was able to induce this effect with virtually 100% efficiency. The effectiveness of ALS in suppressing cellular immune-response has been shown in many viral infections, such as vaccinia, herpes simplex, yellow fever, lymphocytic choriomeningitis, murine leukemias, adenovirus type 12, polyoma, although the mechanism of action of ALS was not fully resolved. Moreover, the marked amplification of reovirus infection by ALS may be similarly explained to a consequence followed by suppression of the cellular immunity. If this is the case, following considerations may be not worthless. First, different susceptibility to reovirus infection at different ages of mice in the aspect of appearance of acute disease seems to indicate the difference between the maturation of cell-mediated immune mechanism in young infant mice and that in older mice. The 5-day or younger mice in the present studies may be in immature state for the induction of such cell-mediated immunity, but the 7-day or older mice are possibly in mature state in this sense. Secondly, at least a part of mechanisms of both the inapparent infection in older mice and recovery from the acute illness in younger mice, in the case of reovirus infection, can be explained by the action of the cellular immune-mechanism. Previous data have indicated that the productions of humoral antibody and or interferon in vaccinia infection are not suppressed by ALS. In the present study, a suppression of humoral antibody to reovirus infection by ALS was not evident, although an assay of interferon was not done. Taken together, it may be assumed that the cellular immune-mechanism may play an important role on the recovery from or the prevention of reovirus infection in vivo. However, direct evidences of the cellular immunity expected in this infection must be examined by further studies, especially by aid of in vitro experimental procedures. Studies in this line are under progress.

INFLUENCE OF ALS ON THE ANTIGEN-REACTIVITY OF BONE-MARROW CELL (GUINEA PIG)

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Recent evidence suggested the bone marrow constitutes a major souce of stem cell for humoral antibody formation. The function of the bone marrow in the immune responce has been analyzed by cell transfer system in vivo and cell culture system in vitro. On the other hand, several investigations have demonstrated that hypersensitivity to staphylococcal infection. In guinea pigs, the susceptibility of this hypersensitivity was transfered by lymphoid cells from hyperimmunized animals. The cell wall of most of S. aureus strains contains a protein which precipitates in agar with normal human globulin and with sera from guinea pigs. There are two major groups somatic antigens in staphylococci, protein A and ribitol teichoic acid. Protein antigen A has a property to bind Fc-fragment of γ-globulin. Also, protein A released histamine from leukocyte, but teichoic acid or teichoic acid-mucopeptide complex did not.
The purpose of this investigation was an observation against the influence of ALS on the antigenicity of staphylococcal protein A and the induction of delayed hypersensitivity in vitro. We used partially purified protein A as antigen from Cowan I, and 406 strain which checked the biological character precisely before. The influence of ALS was examined on the guinea pigs ileum in vitro, and skin reaction in vivo. The cultured bone-marrow cell from which collected immunized guinea pigs, were employed to observe the affection of ALS in a delayed type hypersensitivity induced by protein A. The reactivity of the cells were estimated by the methods of the measurment of LDH activity in cells and of histamine or serotonin release from cells.

Materials and methods :

Animals ;

Guinea pig (Hartley) of both sexes weighing 200-300 g used. Rabbits (New Zealand White) of male weighing 2-3 kg were used.

Preparation of staphylococcal crude and partially purified protein A :

Heat extraction was performed as described by Jensen (1959), in 1/15 M phosphate buffer, pH 5.9 in boiling water for 60 min, followed cooling to 4 C. The supernatant was adjusted to pH 3.0 with 0.1 N HCl and preciptates was collected by centrifugation, at 8,000 x g, for 20 min, and dissolved in phosphate buffer pH 5.9. Ethanol added to the solution at a final concentration of 2.0 % in solution and the resultant precipitate was collected. After the precipitate dissolved in phosphate buffer, the final solution was dialyzed against distilled water at 4 C ovrnight, and stocked samples as the crude protein A by lyophilization. The lyophilized material was gel filtrated by a column of Sephadex G-100, the fractionates were equibrated and eluted with 0.1 M NH_4HCO_3. The elutants were pooled and concentrated by ultrafiltration employing collodion bags (Sartorius Membranfilter, No. 13200).

ALS :

Preparation ;

Rabbits were immunized with 200-500 million guinea pigs thymus or peripheral white cell injected with complete adjuvant intramuscularly, once a week for 3 or 4 weeks. The rabbits were bled 7-10 days after the final injection. The individual sera were pooled, and the pooled serum was absorbed with normal guinea pig RBC and RBC-stroma. The haemagglutination titer showed negative value after the treatment of twice absorp-

tion by a standard method on haemagglutination. The antibody titer of serum, as determined in a cytotoxicity test employing guinea pig peripheral leukocytes was 64 (reciprocal titer). The serum was frozen at -70 C until use.

Cell :

Preparation ;

Guinea pig bone marrow cells were removed by aspiration of the femur and tibias. The cells were suspended in TC-I99 (Difco), and were shaken vigously for 3 min, with glass ball (0.3 cm diameter) into a bottle, and centrifuged at 45 x g for 5 min. The fatty layer was decanted and the cells were suspended in TC-I99. The cell suspension was passed successively through a cotton fiber (No. 50:8). The filtrate was cenrifuged at 50 x g for 5 min, and the packed cells were resuspended in TC-I99. Separating-centrigugation of the marrow cell suspension on the dextran gradient was carried out 25 x g for I0 min. Dextran layers were three different solution (specific gravity I.0560, I.0600 and I.0670 solution). Maximum absolute numbers of bone marrow lymphocytes was found in layer 2 of dextran gradient. This layer should be expected the souce of guinea pig marrow lymphocyte used in all of our experiments.

Incubation and culture ;

The cells separated from guinea pig bone marrow was resuspended in TC-I99 (contained penicillin, streptomycin and I0 % of normal guinea pig serum) to a cell concentration of $I0^7$/ml. The cells were incubated in an atompsphere of 5 % CO_2 in air for adequated intervals at 37.5 C under the designes of our experiments.

LDH activity measurment :

The cells were washed with PBS twice at 2,300 x g for I0 min, and resuspended in tris-HCl buffer (pH 7.4, I/μ 0.05). All cell supernatant was collected by centrifugation, at 230,000 x g for 20 min. The enzyme assay, description of units, and procedure for electrophoresis and detection of LDH isozyme was employed by a method of Starkweather et al (I966).

Cellular observation :

Incubated and cultured cells were tested by trypan-blue inclusion method, and observed May-Giemsa staining. The living and metaphase cells were counted compared with total number of cells.

Measurment of lysozyme activity :

Lyso-plate method was performed by Osserman et al (I970).

Measurment of serotonin and histamine :

The estimation of serotonin and histamine were performed by the spectrophotofluorometric assay by Shore (I960) and Snyder (I965).

Test on isolated guinea pig ileum :

A detail of our experiment for Shultz-Dale reaction was performed by modified method of Farmer (I937).

Results :

The yield of protein A after chromatography on DEAE-Sephadex was approximately 50 % of whole cell volume and about 20 fold degree of purification was achived. Crude protein A caused to contract isolated ileum from guinea pigs which were active immunized by protein A and passive immunized by anti-protein-A serum, but the protein A extracted from 406α strain did not cause contraction, except protein A from Cowan I.

The protein A from Cowan I showed that desensitization of locale anaphylaxis by ileum of guinea pig which immunized by protein A from 406α strain. In the guinea pigs immunized by crude protein A, both before and after successively pretreated by ALS, isolated guinea

pig ileums were contracted by protein A, but more slightly than only in guinea pigs immunized by protein A. Antigenicity of each protein A from Cowan I and 406 have slightly differences on reactivity in vitro.
The LDH activity of normal BMC increased slightly by the addition of a small amount of protein A to the culture medium. When the animals were preimmunized by protein A, the activity was not changed. The release of histamine and serotonin from leukocyte and BMC showed in guinea pigs sensitized by protein A from Cowan I, not showed in normal. The reactivity of rabbit bone-marrow cells was different between normal and immunized animals. The cell-reactivity and responce to sentization could be considered as aparted mechanism treated ALS did not react to a protein A in this experiments.

Discussion :

Protein A was isolated from S. aureus and shown to precipitate most of a pooled normal γ-globulin preparation. Titration of antigen was carried out by gel diffusion in agar slides. In preliminary titration, a two fold serial-dilution gradient was used. For more accurate titration other dilution series were set up.
Antigen titration was carried out in order to the calculate total activity of antigen A, recovered after each purification step. The total of antigen A is expressed in antigen units (AU) and calculated from the equation dilution x volume undiluted antigen A. The activity is expressed as dilution/mg protein per ml.

Normal rabbits bone-marrow cells has been demonstrated according to their immunological reactivity in vitro. Rabbit bone marrow serves as the prime souce of antigen-reactivity cell and the only with respect to antigens. In mouce, the bone marrow did not serve as a souce of the antigen reactivity cells and was the antibody forming cells. The function of the guinea pig's bone marrow has not been analyzed on a view of the antigen reactive phenomenon, in vivo and in vitro.
White (I969) reported that histamine and other pharmacological active peptides mediate the altered vascular permaeability forced in localization acute hypersensitivity reactions. Cowan I antigen also released leukocyte histamine, but teichoic acid and acid-mucopeptide complexs of chemically extracted teichoic acid did not.
Antilymphocyte serum had not effect of the action of α-lysin, but reduced the inflammatory responce to cotton dust and to turpentine. Immunized mice trated with antilymphocyte serum behaved more like unimmunized mice, but did not develop lesion. (Glynn, I969). Stalenheim (I967) showed that Arthus-like reaction in rabbits are produced by the interaction of γ- globulin and protein A.
By our results, desensitization by protein A from Cowan I was observed by locale anaphylaxis. Antigencity of protein A was effected purity of protein A and responce of guinea pig, but influence of ALS on immunized guine pig showed same reaction of ALS non treaed guinea pig. We used LDH activity measurment and isozyme pattern analysis as a marker for the reactivity of cells, and the affection of ALS on the cells. The affection of ALS against the functions of bone-marrow cells was considered commonly on the haematopoietic activity. It might be necessary to examine the immunological phenomena of their reactivity under the influence of ALS.

Summary :

I. Hypersensitivity was induce by intramuscular injection of protein A prepared from the Cowan I strain (S. aureus) in guinea pigs.
2. The induction was confirmed employing in vivo and in vitro tests.
3. The affection of ALS against a responsibility of hypersensitivity-induction was observed in animals which were treated by ALS at pre- and post-term of antigen injection.
4. The antigen-reactivity of cultured bone-marrow cells from which collected normal and immunized animals was examined by intracellular activity and histamine or serotonin release from cells.
5. The influence of ALS on the reactivity of cells was demonstrated in a variety for the phase of the antigen sensitization.

Lymphoid cell surface antigens and cell-mediated immunity

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Introductory remarks : Allogeneic inhibition and cell-mediated immunity

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同種腫瘍細胞に対する試験管内細胞性免疫反応

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In Vitro Cell-Mediated Immune Reaction against Allogeneic Tumor Cells.

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Recent progress in the study of cell-mediated immune reaction in vitro has revealed that in the first step of the reaction sensitized lymphoid cells attach to the antigenic cells or react with antigen and then release a cytotoxic substance which acts to mammarian cells without immunological specificity. Specificity of the reaction may be mediated by the first step. However, many detail points in the mechanism are still remained uncertain.

In order to approach the mechanism we have established a several sensitive cell-mediated immune reaction systems in vitro. Host-antigen combinations are Donryu rat-Yoshida sarcoma or -leukemia cells of the strain, and inbred strains of mouse -various allogeneic leukemia cells. By using the systems we tryed to demonstrate the following points in this paper: 1) Kinetics of target cell destruction, 2) similarity and diversity of sensitivity of target cells against humoral and cellular immune reactions, 3) differences of mode of action in humoral and cellular immune reactions, and 4) possibility of the presence of diffusible cytotoxic factor in the reaction systems.

Materials and Methods

Animals : Donryu and ACI/N rats, and C57BL/6, A, and BALB/c mice were used.

Tumor cells : Yoshida sarcoma, DBLA1 (Donryu rat leukemia), RADA1 (A), BALB/c RL2, and ELA (C57BL) cells were serially passed in the syngeneic animals and tumor cells harvested from the ascites were used for immunization and for test cells.

Immunization and preparation of lymphoid cells : Except for the immunization of Donryu rats with Yoshida sarcoma cells, animals were injected with a small number of tumor cells and subsequently with a larger number of tumor cells. Peritoneal exudate cells and spleens were obtained from immune animals 7 to 10 days after the last immunization. Peritoneal lymphocytic cells (PLC) were fractionated from peritoneal exudate cells by removing macrophages with a glass or plastic tissue culture bottle. Spleen cells were prepared in the usual manner.

Cytotoxic test : Eagle or RPMI1640 supplimented with 15% inactivated fetal calf or rat serum was used for the culture medium. The known numbers of target tumor cells and aggressor lymphoid cells were mixed. Usually 0.5 ml of the cell mixture was placed in a test tube and incubated at 37°. Target cells remaining after a reaction were counted visually under a phase-contrast microscope in a hemocytometer and in some experiments they were measured by the retention of radioactivity in viable target cells which had been labeled with ^{3}H-uridine.

Results

a) Extent of cytotoxic reactions measured by ^{3}H-uridine labelling method : When ^{3}H-uridine labelled tumor cells were employed for humoral or cellular cytotoxic reaction, retention of the radioactivity in target tumor cells was parallel to the number of living tumor cells counted visually. (Fig. 1) Releasing rate of radioactivity from the destructed cells was higher than the rate in known methods such as ^{51}Cr or ^{3}H-thymidine labelling. One

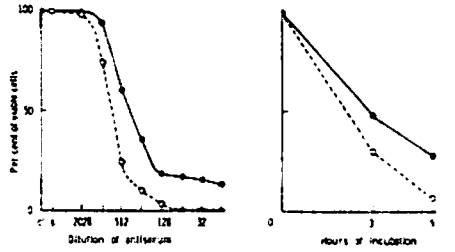
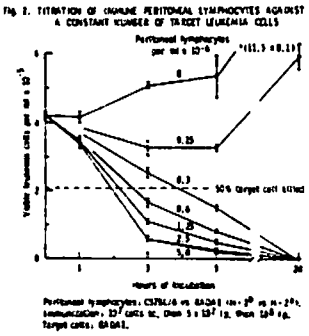


Fig. 1 a. Titration of C57BL/6 vs BADA1 serum against BADA1. ○-○ visual count, ●-● ^{3}H-uridine method. b. Cytotoxic test of PLC from C57BL/6 vs BADA1 against BADA1. Same as in a.

disadvantage of this method was in the reutilization of released radioactivity by the surviving target cells. However, in such rapid immune reactions as humoral cytotoxic reaction or the present cell-mediated immune reaction, the possibility was minimized.

b) Kinetics of target cell destruction : Continuous observation of cell-mediated immune reaction in a microchamber showed that in the case of Donryu rat-Yoshida sarcoma system one tumor cell was destroyed by more than 2 sensitized PLC adhering to the cell, whereas PLC incubated for 24 hrs or more destroyed one Yoshida sarcoma cell by one cell. In mouse-allogeneic leukemia system, the ratio was one to one, and after the destruction of a leukemia cell the aggressor PLC adhering to the leukemia cell was still viable, suggesting a possibility that the PLC could act to another target leukemia cell. Time required for the destruction of a target tumor cell was usually shorter than 2 hrs in these experiments. The result of titration of sensitized PLC against target leukemia cells was shown in Fig. 2. The time for destruction of target cells was directly related to the ratio between numbers ofsensitized PLC and target leukemia cells. Per cent of target cells adhering to PLC was also related to the ratio.



c) Similarity and diversity of sensitivity of leukemia cells against humoral antibody and sensitized lymphoid cells : Reactivities of leukemia cells of various H-2 specificities to immune serum and to sensitized PLC obtained from the same donor animals were studied. Leukemia cells of the same strain as immunizing cells, 3rd party strains which had 5 out of 8, 5 out of 6, and 2 out of 6 H-2 specificities freign to the immune hosts were killed rapidly by the sensitized PLC. Whereas leukemia cells of 3 rd party strain having 1 out of 4 H-2 specificity were not effected by the sensitized PLC, although the cells were destroyed by the serum from the same immune animals. Susceptibilities of leukemia cells to immune serum and to sensitized PLC were not always parallel, e.g. H-2^{b} vs H-2^{k} serum showed the same cytotoxic titer to H-2^{k} and H-2^{d} leukemia cells, however, sensitized PLC destroyed the former leukemia cells much faster than the latter. These data may suggest that the antibody reactive site on leukemia cells to humoral antibody and to sensitized lymphoid cells may be different. This assumption was substantiated by blocking experiments in humoral and cellular immune reactions; mouse leukemia cells of known H-2 were homogenized and the nuclear part was removed by centrifugation. The supernatant fraction was centrifuged at 100,000 G for 1 hr. Precipitated segement was suspended in culture medium, and a part of the suspension was added to immune serum or sensitized PLC. After an incubation for 30 min at 37°, to the mixture was added target leukemia cells, and cytotoxic tests were carried out in the usual manner. Result represented in Fig. 3 showed that humoral

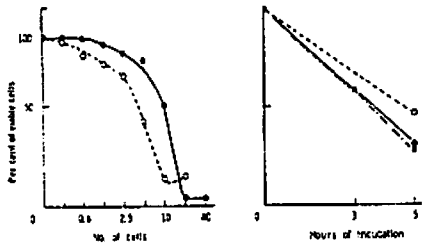


Fig. 3 a. Blocking test of CSTBL/6 vs RADA1 serum by ○—○ viable RADA1 cells, and by ●—● the fragment

b. Blocking test of PLC from CSTBL/6 vs RADA1 against RADA1 by viable RADA1 (5×10^5) ○—○ , and by the fragment (2.5×10^6) ●—● . ●···● Control.

immune reaction was blocked by the cell fragment, but cell-mediated immune reaction was not effected.

A further experiment is required to know whether the abolishment of reactive site to sensitized lymphoid cells after homogenization of the antigenic cells is due to the destruction of the site during the fractionation process or unsuitableness of the experimental method.

d) Different mode of action in humoral and cell-mediated immune reactions : In cell-mediated immune reaction, close contact between target cells and aggressor lymphoid cells is a necessary step for the reaction. However, mediator of the attachment has not been clarified. From the following experiment humoral antibody (cell-fixed antibody) may be ruled out as the mediator; papain treatment of mouse leukemia cells which had been sensitized with anti-H-2 immune serum removed the antibody from the cells, whereas the same treatment of sensitized PLC did not affect the ability of adhering and the reactivity.

It has been well known that complement is not required to a cell-mediated immune reaction. Addition of trypan blue to a humoral immune reaction system abolished the target cell lysis, since the dye inhibit the binding of complement to antigen-antibody complex. However, trypan blue did not inhibit a cell-mediated immune reaction. So that complement supplied from aggressor lymphoid cells, even there is a possibility, may not take part in the cell-mediated immune reaction.

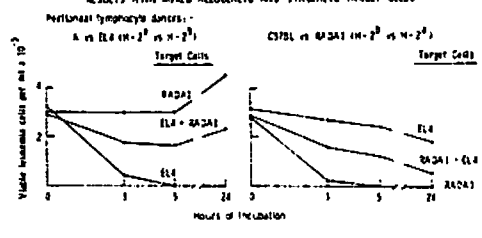
e) Possibility of the presence of a diffusible cytotoxic factor in the present cell-mediated immune reaction system : Two types of experiment were carried out; 1) mouse leukemia cells of immunizing strain and leukemia cells syngeneic to the aggressor PLC were incubated with sensitized PLC in a test tube. 2) To a monolayer of embryonic fibroblasts were added leukemia cells having a different H-2 genotype and sensitized PLC or spleen cells which were obtained from mice immunized with either the leukemia cells or cells syngeneic to the fibroblasts. Characteristic feature of these two systems are in the close distances from sensitized lymphoid cells to other two types of target cells, and if any non-specific cytotoxic factor is released from the sensitized lymphoid cells, it may act to two types of target cells in a high concentration. As demonstrated in Fig. 4 and Table I, only

Table 1. Mixed Culture of Fibroblasts and Leukemia Cells with Lymphoid Cells Sensitized to one Strain of the Cells

	C57BL F. BALB/c vs EL4 PLC	C57BL F. C57BL vs RADA1 SPC* or PLC**		A/He F. C57BL vs RADA1 PLC	Donryu F. Don. vs DBLA1 PLC
	RADA1	RADA1		RADA1	DBLA1
F	100[1] 100[2]	100* 100*	100**	100	100
F+L	11 29	128 110	93	24	134
F+T	106 108	175 135	125	109	145
F+L+T	83 40	121 94	95	91	144
T	100 100	100 100	100	100	100
F+T	96 119	98 171	139	146	92
F+L+T	93 86	7 46	50	19	41
L+T	87 78	3 10	32	17	53
cult. t.	48 48	24 48	24	48	48 hr

Figures in table show the per cent no. of viable cells by ^{3}H-uridine method.
F : fibroblasts, L : lymphoid cells, T : tumor cells. Prime symbole represents treated test cells.
1) RADA1 : 2.5×10^5, 2) : 6×10^6.

Fig. 4. SPECIFICITY OF H-2 IMMUNE REACTIVITY OF PERITONEAL LYMPHOCYTES IN VITRO: RESULTS WITH MIXED ALLOGENEIC AND SYNGENEIC TARGET CELLS



the target cells which have antigenicity to sensitized lymphoid cells were killed by the sensitized lymphoid cells, whereas antigenically unrelated were not effected

Thus, it is concluded that at least in the present in vitro systems of cell-mediated immune reaction, non-specific cytotoxic factor like Lymphotoxin will not be released from sensitized lymphoid cells in a sufficient amount to act cytotoxic.

Local graft-versus-host reaction in rats.

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The inoculation of lymphoid cells from inbred parental strain rats, when the cells were inoculated in the subcapsular space of the kidney of F1 hybrid rats, was followed by, macroscopically, the increase in weight of the inoculated kidney, and microscopically infiltration of lymphoid cells and destruction of renal parenchyma in the host. This was defined as local graft-versus-host reaction(GVHR) by Elkins.

In a series of his reports, following conclusions were claimed. 1) The degree of this reaction depended upon the origin of inoculated lymphoid cells. 2) Host origin cells were required essentially as antigens for the development of this reaction. Renal parenchymal cells of hosts were merely bystanders involved in the reaction promoted with transplanted lymphoid cells and host mononuclear cells. 3) This reaction was demonstrable , when the donor and host were incompatible as to the major histocompatibility antigens. Effect of immunization was not demonstrable.

The purpose of this report was to investigate the origin, nature and role of donor cells which initiate this reaction, as well as of host origin cells. The role of primed cells inthis reaction was also investigated.

Inbred rats, Wistar-King-Aptekman (WKA), Tokyo, Buffalo and Fischer were obtained from Laboratory of Experimental Animals, Hokkaido University, Sapporo. These rats were controlled by allelic genes of R-locus, which is the major histocompatibility locus of rats.

According to the description of Elkins, 0.2 ml. of lymphoid cell suspensions prepared in Hanks' solution were inoculated into the subcapsular space of the left kidney via a 30 gauge needle.

Usually host animals were sacrificed at 7 days. The index of weight gain of injected kidney was calculated from the weight ratio of inoculated to contralateral kidney (Ki/Kc).

Accumulation of mononuclear cells in the subcapsular space and a tendency of its infiltration to renal parenchyma was evaluated as positive GVHR. The degree of invasive activity was devided into 3 groups according to Elkins' criteria, namely Grade 1; non-invasive reaction, Grade 2; mononuclear cell infiltration with invasive tongue and Grade 3; extensive invasive-destructive reactions.

Anti-lymphocyte serum (ALS) was obtained from immunization of rabbits with rat thymocytes. Pooled sera were absorbed twice by rat red blood cells. ALS thus prepared, showed 1 : 1280 leukoagglutination titer and 1 ml of the serum was injected intraperitoneally.

Lymphoid cells of WKA rats from different sources were inoculated beneath the renal capsules of (Tokyo x WKA)F1. Inoculation of peritoneal cells, thymocytes and bone marrow cells gave weak or no GVHR, compared to the strong reaction by inoculation of spleen cells.

Two to5 x 10^7 spleen cells harvested from the donor WKA at various intervals after i.p. injection of 1 ml

of ALS, were inoculated beneath the renal capsules of F1 hosts.

There were neither mononuclear cell infiltration under the renal capsules nor nodular lesion, when the injected cells were obtained from the donors injected with ALS within 8 days.

The recovery of immunosuppressive effect was demonstrable from the donor injected with ALS 13 days before. Ki/Kc ratio, however, did not increase in this case.

Suppression of GVHR was more manifested when the donor of spleen cells were thymectomized beforehand in addition to the injection of ALS. Transfer of 4-5 x 10^7 spleen cells from the donor WKA which had been thymectomized and injected with ALS 21 days previously, gave little mononuclear cell infiltration in the injected kidney.

These results can be well explained if the thymus-dependent cells of the donor initiate this reaction and are the target of ALS.

By pretreatment of donor with ALS, thymus-dependent cells in the animals were possibly destroyed for a period and later, because of the presense of thymus, these cells were considered to be recovered in peripheral parts and could initiate GVHR. By the use of thymectomy, the recovery of thymus-dependent cells , once destroyed with ALS, would not occur so that suppressionof GVHR was more intense and persistent.

Different GVH reactivity of various lymphoid cells seems to indicate different distribution of thymus-dependent cells in the tissue or organ of the donor.

While thymus-dependent cells of the donor seem to be essential for the initiation of GVHR, this reaction was greatly suppressed by the prior irradiation (1,000R) of hosts. When 5 x 10^7 of spleen cells from the donor WKA were injected into heavily irradiated (WKA x Tokyo)F1 rat, increase in Ki/Kc ratio as well as invasive activity were greatly suppressed. This result indicates that host origin cells are also essential for the development of this reaction.(Table 1)

When the host F1 were thymectomized and treated with ALS 14 or 21 days previously, the degree of this reaction was not different from that of normal hosts. Because there could be few thymus-dependent cells in the thymectomized and ALS-treated animals in this period, this result indicates that large number of host origin cells in the reaction site are not thymus-dependent in nature.

It was observed that the decrease in Ki/Kc ratio and invasive activity was prevented by i.v. injection of bone marrow cells syngeneic to the host strain rats soon after lethally irradiation of hosts(Table 1).

This result, in addition to the fact that host origin cells are thymus-independent, indicates that host origin cells in this reaction will appear in the reaction site directly from the bone marrow.

GVHR did not decrease so markedly by the inoculation of spleen cells from WKA sensitized to Tokyo antigens into 1,000R irradiated (WKA x Tokyo)F1, compared to the reaction by inoculation of non-sensitized spleen cells (Table 1).

Since the reaction with spleen cells from nonspecifically sensitized WKA did not recover, primed cells seem to react specifically to the renal parenchyma of hosts.

Host mononuclear cells seem to be unnecessary for the development of this reaction with sensitized donor cells, but indispensable for the reaction with non-sensitized donor cells in the course of antigenic recognition.

Another important poit from this result is the fact that the effect of immunization is demonstrable even when the donor and host are histoincompatible.

Various doses of spleen cells from WKA were inoculated beneath the renal capsules of (WKA x Buffalo)F1, which were major R-incompatible from donor strain rats.

At the dosage ranging from 10^7 to 5×10^7 cells from normal donors, the degree of this reaction increase in proportion to the number of cells inoculated. The reaction reached plateau level at the dosage more than 5×10^7.

On the other hand, plateau was obtained at the dosage more than 2×10^7 by inoculation of spleen cells from WKA sensitized to Buffalo antigens.

At the dosage of 5×10^7, spleen cells from sensitized donors gave about equal reaction to that by normal donors, whereas at 2×10^7 there was marked difference between the reaction with spleen cells from these two donors, Effect of immunization was demonstrable at the plateau level.

In the time-course experiment, strong reaction was not obtained at the early stage by inoculation of sensitized spleen cells to compare with the reaction of nonsensitized spleen cells, disappointing, because primed cells were thought to react diredtly to the host renal parenchyma.

The difference between the reactions by these two cells began to be apparent 5 Or 6 days after inoculation of spleen cells.

F1 hybrid hosts received an intraperitoneal injection with 1 ml of ALS at various intervals after inoculation with 5×10^7 spleen cells from either sensitized or nonsensitized parental donor.

The reaction did not decrease so markedly when hosts were given ALS 6 hours after inoculation of the primed cells In cortrast, the reaction decreased markedly when the hosts were injected with ALS 6 Or 20 hours after inoculation of non-primed cells.

These results suggest that there are marked difference as to the function between primed and nonprimed lymphoid cells although there are no morphological difference between those two

Table 1 Effects of host irradiation(1,000R) on local GVHR.

donor state	host state	rat no.	Ki/Kc	S.E.
normal	normal	3	1.41	0.09
normal	irrad.	29	1.10	0.01
spec. immune	irrad.	10	1.31	0.05
nonspec. immune	irrad.	6	1.09	0.04
normal	irrad.& bone marrow cells i.v.			
	1.4×10^8	3	1.59	0.14
	1.2×10^8	2	1.09	0.14
	1.5×10^8	3	1.39	0.06
	3×10^8	3	1.27	0.05
	2.3×10^8	3	1.20	0.09

sources of the cells.

At the same time, these results suggest that ALS acts on unprimed thymus-dependent cells rather than on on differentiated primed cells.

In summary, then, the following comments may be mentioned as to the nature of donor and host cells involved in the local GVHR of rats.

Donor lymphoid cells which initiate this reaction are thymus-dependent and seem to request host mononuclear cells rather than host renal parenchyma for antigenic recognition.

Host mononuclear cells participating in the reaction are thymus-independent.

Primed donor lymphoid cells produce " effector cells" or "memory cells", which may not be distinguished from nonprimed lymphoid cells at least in light-microscopic morphology but may be functionally characteristic so that these cells react directly with renal parenchymal cells and are insensitive to ALS.

ニワトリの血液型とGVH反応

藤尾芳久（名大・農・家畜育種）

Relationship between Blood Group Gene B and Graft-versus-host Reaction Activity in the Chicken. YOSHIHISA FUJIO (Laboratory of Animal Genetics, Faculty of Agriculture, Nagoya University)

The splenomegaly has been recognized as an expression of the immuno-reaction to foreign antigens. It was shown by many workers that the splenomegaly in the chick embryo was the result of a graft-versus-host(GVH) reaction, in which the blood group gene B plays an important role, namely, splenomegaly was observed only in the case that the host had the antigen controlled by the gene B which was not possessed by the graft(Jaffe and McDermid 1962; Schierman and Nordskog 1963; Gilmour 1963; Crittenden et al. 1964). Schierman and Nordskog(1963) indicated that the gene B controlled the formation of foci on the chorioallantoic membrane of chick embryos as well as splenomegaly after inoculation with adult chicken leucocytes.

The present work was designated to determine whether the gene B controls the formation of foci on chorioallantoic membrane as well as splenomegaly, and if it is case, to make quantitative analysis on the effect of the gene B.

The eggs and bloods used for the present work were obtained from three inbred strains of chickens and their F_1 hybrids. These three inbred strains were Black Minorca C(BM-C), Nagoya B(NG-B), and CON. CON was originated from a cross between White Cornish and New Hampshire. The birds of these strains were homozygous for the genes determining blood group antigens as shown in Table 1.

Table 1. The blood group antigens of strains of chicken

Strain	Blood group system									
	A	B					D	R	Unknown	
	P	A	B	G	K	M	Q	R	Q'	S
BM-C	-	+	-	-	-	-	+	-	+	-
BM-C	-	-	+	-	-	-	+	-	+	+
CON	-	-	-	-	+	-	+	-	-	-

Peripheral blood leucocytes from adult birds were deposited on the chorioallantoic membrane of 13-day embryos. The inoculation consisted of 0.1 ml of a leucocyte suspension prepared by resuspending the buffy layer of donor blood in Tyrode's solution to give a concentration of 1.0×10^7 leucocytes per ml. After four days, counts were made of the number of foci produced. At the same time, the spleen of the host embryo was weighed.

The results indicated that production of foci was principally due to the blood group gene B incompatibility, that is, production of foci was observed when the embryo had a gene B(an antigen) which was not possessed by the leucocyte donor(Table 1). These foci were white and visible as shown in Fig. 1. No foci were produced when donor and host

Table 2. Focus formation and spleen weights of blood grouped host embryos inoculated with leucocytes from blood grouped donors

Combination	Group	Donor genotype	Host embryo: Genotype	Host embryo: No.	No. of focus	Mean spleen weight(mg)
I	1	B^AB^A	B^AB^A	18	0.2 (0 - 2)	13.6 (10 - 17)
		B^BB^B		13	27.3 (25 - 31)	25.5 (24 - 33)
		B^AB^B		14	0.4 (0 - 2)	14.3 (11 - 18)
		Control		20	0.0	12.5 (8 - 15)
	2	B^AB^A	B^BB^B	12	5.3 (4 - 7)	16.8 (16 - 19)
		B^BB^B		12	0.2 (0 - 2)	12.2 (11 - 13)
		B^AB^B		13	0.3 (0 - 2)	11.8 (11 - 13)
		Control		10	0.0	11.5 (9 - 13)
	3	B^AB^A	B^AB^B	12	3.8 (2 - 6)	15.1 (13 - 18)
		B^BB^B		10	9.7 (6 - 17)	20.4 (16 - 24)
		B^AB^B		10	0.0	14.7 (12 - 15)
		Control		11	0.0	13.6 (11 - 15)
II	1	B^BB^B	B^BB^B	12	0.2 (0 - 2)	12.2 (11 - 13)
		B^KB^K		14	9.3 (7 - 12)	15.0 (14 - 18)
		B^BB^K		13	0.4 (0 - 3)	11.2 (8 - 14)
		Control		10	0.0	11.5 (9 - 13)
	2	B^BB^B	B^KB^K	12	22.5 (20 - 27)	19.0 (15 - 20)
		B^KB^K		15	0.4 (0 - 2)	10.4 (7 - 14)
		B^BB^K		14	0.4 (0 - 3)	10.5 (8 - 13)
		Control		10	0.0	10.6 (9 - 13)
	3	B^BB^B	B^BB^K	16	13.0 (9 - 14)	19.2 (15 - 23)
		B^KB^K		12	4.3 (3 - 6)	13.3 (11 - 17)
		B^BB^K		20	0.4 (0 - 2)	12.2 (9 - 14)
		Control		14	0.0	11.2 (10 - 13)
III	1	B^AB^A	BABA	18	0.2 (0 - 2)	13.6 (10 - 17)
		B^KB^K		20	15.3 (12 - 19)	23.6 (19 - 28)
		B^AB^K		20	0.3 (0 - 2)	13.3 (9 - 17)
		Control		20	0.0	12.5 (8 - 15)
	2	B^AB^A	B^KB^K	15	23.4 (18 - 27)	27.8 (24 - 33)
		B^KB^K		15	0.4 (0 - 2)	10.4 (7 - 14)
		B^AB^K		12	1.0 (0 - 3)	11.4 (8 - 14)
		Control		20	0.0	10.6 (9 - 13)
	3	B^AB^A	B^AB^K	16	10.8 (9 - 15)	19.8 (17 - 24)
		B^KB^K		20	6.0 (4 - 9)	15.6 (12 - 19)
		B^AB^K		18	0.6 (0 - 3)	12.6 (8 - 16)
		Control		12	0.0	10.0 (8 - 12)

Fig. 1. Formation of foci on chorioallantoic membrane. BM-C(B^AB^A) host embryo was inoculated with 1.0×10^7 leucocytes from CON(B^KB^K) donor.

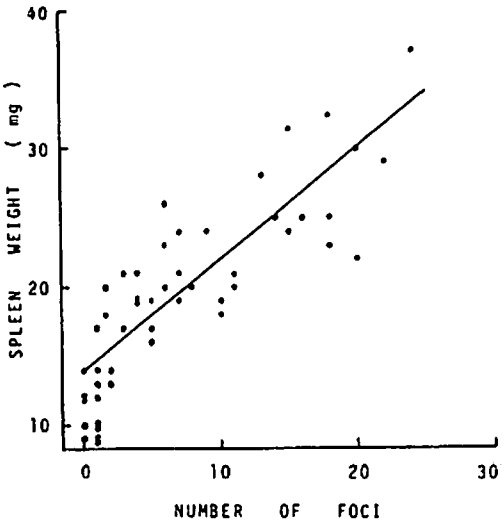

Fig. 2. Correlation between the number of foci produced and the mean spleen weight.

were B-compatible.

Further evidence for the gene B control of the GVH reaction was shown by splenic enlargement in the B-incompatible embryos. A linear correlation was observed between the number of foci and the mean spleen weights in the B^AB^A hosts which were inoculated with various number of leucocytes from B^KB^K donor as shown in Fig. 2.

The mean focus number for the B-incompatible combinations of B^BB^B donor and B^AB^A host, B^BB^B donor and B^KB^K host, and B^AB^A donor and B^KB^K host were greater than those for their reverse combinations, respectively(Table 2). Similar relations between genes were observed in the activity of focus formation, when heterozygous F_1 embryos were inoculated with their parental homozygous leucocytes. A possible explanation of these differences, following a template theory, is that the larger the differences in antigenic factor between donor and host stimulate the more proliferation of donor cells. From viewpoint of elective theory, on the other hand, such interpretation may be possible that the leucocyte inoculum from the donor contained different number of immunologically competent cells with preformed patterns.

Further observation of B-incompatible combinations reveals that numbers of foci produced in the homozygous host are twice as much as those in the heterozygous host. The difference in focus number between homozygous and heterozygous hosts may be due to the difference in the production of antigen which depends on the gene dose. From the above result, it may be prefarable to interprete the difference in the production of foci on the basis of templete theory.

Role of Humoral Antibodies in Tumor Allograft Immunity.

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Tumor allograft immunity has been investigated using transplantable mouse plasma cell tumor X5563, which was originated from a C3H/He mouse. A method has been developed to follow the tumor growth in vivo by estimating quantitatively the γ_{2a} globulin produced by the X5563 tumor cells. For this purpose, antiserum against an idiotypic specificity of the X5563 γ_{2a} globulin was prepared by immunizing rabbits with the Fab fragments of the X5563 γ_{2a} globulin in Freund's complete adjuvant and by absorbing it with normal mouse serum. Upon immunoelectrophoresis as well as upon double diffusion test, the antiserum thus prepared did not show any precipitin lines against normal serum but did show a single precipitin line against serum of X5563-bearing mouse (figure 1). Thus, quantitation of γ_{2a} level in serum of tumor-bearing mice could be successfully achieved by radial immunodiffusion in agar containing the specific anti-X5563 γ_{2a} serum.

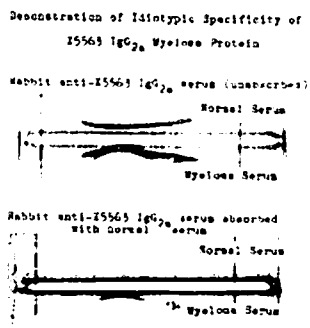


Figure 1.

When the tumor cells were inoculated subcutaneously or intraperitoneally into the syngeneic C3H mice, the X5563 γ_{2a} globulin level in serum increased exponentially in the lapse of time after inoculation of tumor cells until the death of host. Similarily, the increase of the X5563 γ_{2a} globulin level was observed in serum of the allogenic ddO mice inoculated with the tumor. Usually, the γ_{2a} level could be detected as early as 4 days after inoculation of the standard test dose of 5 x 10^6 tumor cells, reached maximum after 10 to 14 days, and followed by the gradual decrease. Less frequently in the ddO mice inoculated with the tumor, the X5563 γ_{2a} level as well as the tumor size continued to increase until the death of host. Parallel relationship was observed between the γ_{2a} globulin level in serum and the tumor size in diameter after subcutaneous inoculation (figure 2). These results indicate that the changes in the γ_{2a} globulin level reflect the fate of tumor in host and therefore, are indicative of the tumor growth and regression.

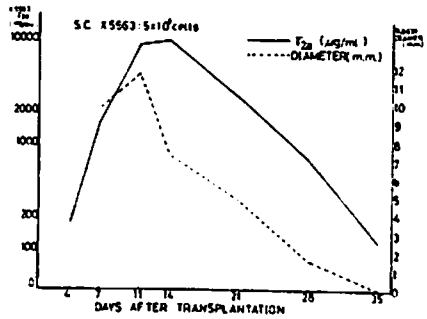
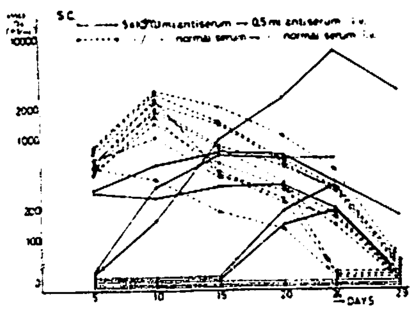


Figure 2.

On the basis of these criteria, effect of isoantisera on the fate of the X5563 tumor cells in vivo was studied. The isoantisera were collected from the ddO mice which had been repeatedly inoculated with viable X5563 tumor cells previously. A pooled isoantiserum was used throughout the present experiments.

First, each normal ddO mouse was subcutaneously inoculated with 5 x 10^6 tumor cells suspended in 0.1 ml of antiserum and normal serum as control, respectively, and also injected with 0.5 ml of antiserum or normal serum intravenously immediately after the tumor inoculation. As shown in figure 3, the growth of tumor in each control mouse, which was exponential in its early phases, gradually decreased after 10 days. This was found to be due to the rise of active immunity (not shown in figure). In contrast, the growth of tumor in the most antiserum-treated mice was suppressed.

In the second set of experiments, 5 x 10^6 tumor cells were inoculated intraperitoneally and 0.5 ml of



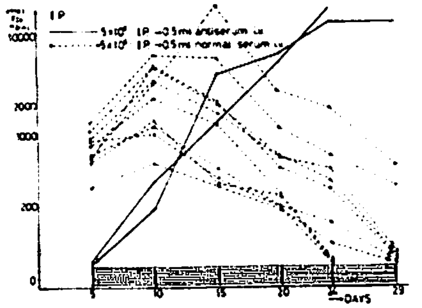


Figure 3.

Figure 4.

antiserum or normal serum was injected intravenously. The results presented in figure 4 were essentially similar to those in figure 3. However, as can be seen in two figures, the tumor cells inoculated intraperitoneally was suppressed more markedly than those inoculated subcutaneously. In the two experiments above described, isoantiserum exerted an inhibitory effect on the tumor growth, especially at an early period after inoculation. However, some antiserum-treated mice showed the delayed but enhanced growth of tumor.

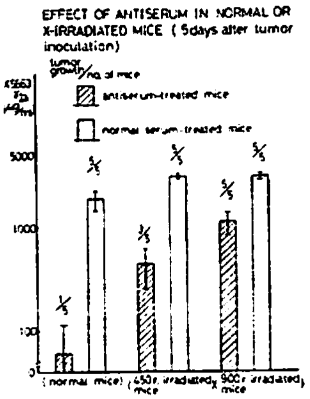


Figure 5.

The suppressive effect of antiserum on tumor growth was not so significant when the recipient mice were irradiated. Tumor cells, suspended in 0.5 ml antiserum or normal serum, were inoculated into the peritoneal cavity of 0, 450 and 900 r X-irradiated mice, respectively. The results are shown in figure 5, and suggest that antiserum alone are not sufficient to destroy target tumor cells and some other factors, susceptible to irradiation, are required for the effectiveness of antiserum.

The tumor growth was also little influenced by antiserum when the tumor cells were placed in diffusion chambers and inserted intraperitoneally into recipient mice. However, the suppressive activity of antiserum for the growth of tumor in diffusion chambers became pronounced when the peritoneal exsudate cells obtained from unimmunized mice, were mixed with the tumor cells (Table 1).

X 5563 growth in millipore chambers in Xray irradiated ddO mice

Exp. No.	No. of mice	Inoculum in* chambers	Treatment†	X5563 γ2a (μg/ml) 7 days after inoculation	Mean value of X5563 γ2a ±SE
1	4	X5563+PEC(2:1)	Immune Serum	0.0.130.0	33±32
2	4	X5563+PEC(2:1)	Normal Serum	180.160.130.160	156±10
3	4	X5563 only	Immune Serum	160.120.140.130	138±9
4	4	X5563 only	Normal Serum	200.160.160.130	163±14

* X5563=10 cells, PEC=5x10^{6} macrophage rich(70%) peritoneal exsudate cells

† i.p. injected 18hrs after placement of the chamber

Table 1.

These observations strongly suggest that host cells, probably macrophages, play an important role on the suppression of tumor growth under the cooperation with the passively administered antibodies. The antiserum was found to be cytotoxic in vitro as judged by release of radioactivities from the Cr^{51}-labeled tumor cells in the presence of complement. However, it is suggested from the results above described that the antibodies may participate in the process of the tumor cell destruction in vivo as opsonins or cytophilic antibodies rather than do as cytotoxic antibodies.

On the other hand, when each dose of 0.05 ml of the same antiserum was injected at the points indicated by arrow mark in figure 6a, a small but significant inhibitory effect on the initial growth of the tumor was

observed. After 2 weeks, however,enhanced growth of the tumor became evident. In contrast with this result, it should be noted that only 2-fold quantity of the antiserum (0.1 ml) caused the complete suppression of tumor growth as shown in figure 6b.

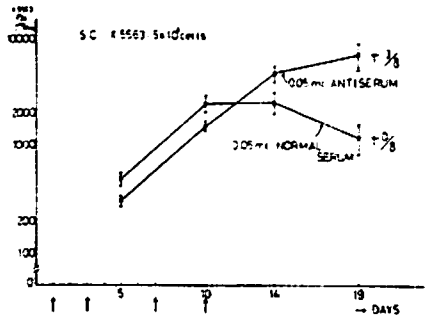


Figure 6a.

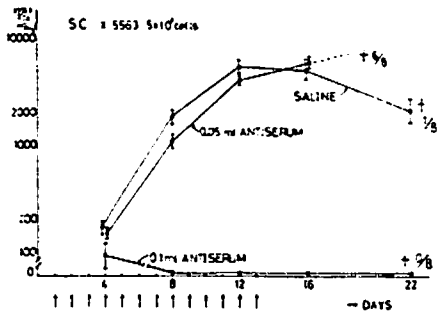


Figure 6b.

The same animals as illustrated in figure 6b (0.1 ml-antiserum- or 0.1 ml-saline-treated mice) were rechallenged intraperitoneally with the tumor cells after the complete regression of primarily challenged tumor. It was found that the immunity against the tumor was developed strongly in the most control mice and poorly in the most mice treated with antiserum as judged by the growth pattern of tumor cells after the second challenge (figure 7). The poor development of immunity in the latter case may be interpreted as the results of either blocking of immunogenic information by antibodies or less antigenic stimulation due to the suppressed tumor growth.

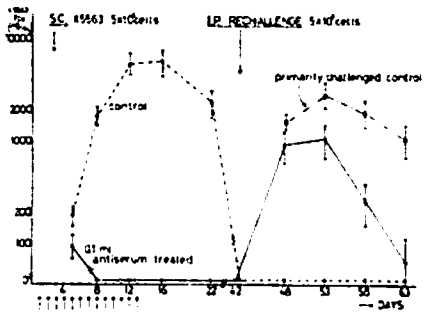


Figure 7.

Enhancement of tumor growth similar to that shown in figure 6, could be also induced in the most of recipient mice which had been pretreated either (1) with passive transfer of immune spleen cells (7 days before tumor inoculation) (2) with active immunization by freeze-thawed tumor cells in Freund's adjuvant or (3) with active immunization by intravenous injection of viable tumor cells (Tumor growth could not be observed in ddO mice when the tumor cells were inoculated intravenously). It is likely that under these pretreatments isoantibodies are produced in the recipients in such quantity as insufficient for complete suppression of tumor growth.

From the results obtained in the present study, it is evident that humoral antibodies affect on the in vivo fate of allografted tumor cells in two conflicting ways; one is suppressive and the other enhancing for the tumor growth. In the former case, the antibodies may accelerate the destruction of tumor cells as opsonins or cytophilic antibodies. In the latter case, the enhanced tumor growth induced by a small dose of antibodies seems to be due to the suppression of the development of host own immunity. The antiserum may destroy a part of tumor cells but the remaining tumor cells continue to grow progressively. The growing tumors may give the increasing antigenic stimulation to the host. Nevertheless, the active immunity of host was appeared to be suppressed since the enhanced tumor growth became evident at the time when active immunity would be acquired unless the antiserum were given. A possible explanation for the suppressed immunity may be blocking the immunogenic information by antibodies.

皮膚反応惹起因子(SRF)並びにマクロファージ遊走阻止因子(MIF)の種属特異性.

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(予研, 結核部)

Species specificity of skin reactive factor(SRF) and macrophage migration inhibitory factor(MIF).

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Most studies on macrophage migration inhibitory factor(MIF) and skin reactive factor(SRF) have been carried out preferably with guinea pigs, although there are increasing number of reports about human MIF. In order to clarify the species specificity of MIF and SRF, we have performed the following experiments.

Materials and Methods

Animals: Random bred Hartley guinea pigs weighing 350-450g, outbred white rabbits weighing 2.5-3.0kg, Wistar rats weighing 180-200g, and outbred ddy mice were used. In addition to these, six different inbred strains of mice were also employed as the recipients of SRF from guinea pigs: these included A,CF1, CBA,DDY,Hairless, and Poor Coated strains.

Lymphocyte culture supernatants: Guinea pigs were immunized with 0.5mg of heat-killed Aoyama-B strain of M.tuberculosis and 4 weeks later challenged intravenously with 0.4mg of live BCG. Ten to 14 days after the challenge the animals were sacrificed to obtain the granulomatously enlarged spleens(mean weight was about 4g). Elimination of erythrocytes from the spleen cell suspension and the lymphocyte cultures with or without tuberculin purified protein derivative(PPD) were performed as described previously(1,2). Duration of the incubation of lymphocytes at 37°C was 24 hours and the final concentration of PPD in culture media was 50 μg/ml. Cell concentration was adjusted to 1.5×10^7 cells/ml. After the incubation the supernatant was separated from cells by centrifugation at 3,000 rpm for 30 min.

Preparation of Sephadex Fraction II from the supernatants: The outline of the procedureis shown in Fig.1. Fifty microgram/ml of PPD were added just before freeze-drying to the supernatant from control culture which was incubated without PPD at 37°C for 21 hours. Fraction II was obtained by the elution of the crude concentrated supernatant through Sephadex G-100 column as indicated in Fig.2. Then it was concentrated by freeze-drying and dialyzed against culture medium(Eagle MEM without serum) before checking its SRF and MIF activity.

Skin test: SRF activity was detected by the intradermal injection of 0.1ml of the material(protein concentration adjusted to 400 μg/ml) in guinea pigs, rabbits and rats. On the other hand, mice received 0.05 ml of the material (protein concentration adjusted to 800 μg/ml).

Histology of skin reaction: Histological examination of skin test sites was performed at 3, 6 and 24 hours after the intradermal injection of the materials. Both control and experimental skin test sites were cut into the sections from three animals of each species at each time. These were stained by hematoxylin-eosin for the histological investigation.

Migration inhibition test: Details of the method have been described previously(2), except that rats and mice were injected intraperitoneally with 10ml and 2 ml of liquid paraffin respectively four days before the harvest of peritoneal exudate cells.

Results

When the concentrated spleen cell supernatant obtained from cultures containing 50 μg of PPD per 1.5 x 10^7cells was applied on Sephadex G-100 column, we have confirmed the results reported by several investigators including those of ours(3,4,5 and 6); MIF activity was found in Fr.II (mean M.W. is around 65,000) and the fraction which had the smaller molecular weight(ca. 12,000). When the same materials were injected into the skin of guinea pigs, inflammatory reactions were induced only by Fr.II(+) as shown in Fig.2. Therefore in the comparison of activities of the materials in each animal species, we have used only the Fr.II throughout the experiment. Fraction II obtained from control spleen cell supernatants was designated as Fr.II(-) and served as control material.

Forty micrograms of the protein of Fr.II(+) and Fr.II(-) were injected into each side of the frank of the same animal. As shown in Table 1, guinea pigs, rabbits and rats showed the strong skin reactions at 6 hours after the injection of Fr.II(+) and none or negligible irritaing reaction remained at the skin test sites injected with Fr.II(-).

Macroscopical observation of these reactions revealed that in both guinea pigs and rabbits the reaction was consisted of erythema and induration which started to appear at 1-2 hours after the injection, reaching the peak of reaction around 6 hours and faded slowly, but still remained moderately at 24 hours after the injection(Fig.3). In the rat the reaction was mainly consisted of induration but erythema was scarcely observed. This reaction was quite similar to that seen in the skin test when the rat was immunized actively. However, the course of reaction in the rat was almost identical to that seen in guinea pigs or rabbits(Fig.4).

In contrast to these species, mice(outbred ddy strain) did not reveal any obsevable reaction when injected with guinea pig Fr.II(+) throughout the observation period. Six different strains of mice, therefore, were skin tested similarly to see if whatever strains of mice could react to the substance. As the result, none of these strains of mice showed any positive reaction at all.

Histological findings: In guinea pigs, rabbits and rats almost the same histological changes were observed; at 3 hours after the injection of Fr.II(+),

there appeared already quite intense cellular infiltration consisted of polymorphnuclear leukocytes(30-50% of infiltrating cells) and mononuclear cells(50-70 %) in the dermis and just above the panniculus carnosus. In the rat, however, it was characterized by the predominant cellular infiltration in the deeper dermis with the less infiltration in the upper dermis.

In these three species, the 6 and the 24 hour reactions had similar aspects as the 3 hour reaction, although the extent of cellular infiltration was increased.

In any strains of mice investigated there was no difference microscopically between the skin test sites injected with Fr.II(+) and those with Fr.II(-).

Macrophage migration inhibition test: Guinea pig MIF, either crude culture supernatant or Fr.II(+), has been tested for the activity to macrophages of rats or mice. As shown in Table 2 , guinea pig MIF could not inhibit the migration of macrophages of rats and mice nevertheless it showed the strong inhibitory activity to the homologous macrophage migration.

Discussion

It can be concluded that SRF of guinea pigs is able to induce the same type of inflammatory skin reaction in rabbits and rats, but not in mice, as in guinea pigs. On the contrary it seems that guinea pig MIF is not able to inhibit the in vitro migration of macrophages obtained from rats or mice.

David and Bloom(7) reported that they could not inhibit mouse macrophage migration with human or guinea pig MIF. However, Thor et al(8) and Rocklin et al (9) have reported that guinea pig macrophage migration could be inhibited with human MIF.

From these facts it may be suggested that SRF and MIF might not be the same substance. This speculation seems to be justified by the fact that the smaller molecular weight substance which showed MIF activity could not induce any inflammatory skin reaction.

Guinea pig SRF could not induce the skin reaction in mice, but one can take it into consideration that, with the exception of a few isolated reports, it has never been possible to observe the characteristic delayed skin reaction in mice even with the active immunization.

In this sense it may be the attractive conclusion that SRF is the possible chemical mediator in the delayed type hypersensitivity. Further investigation should be carried out to clarify the relationship between the SRF and the MIF.

Reference

1) Merler,E. and Janeway,C.A.: Proc.Nat.Acad.Sci., 59:393, 1968

2) Watanuki,M., Yoshida,T. and Hashimoto,T.: Med. Biol., 78:129,1969

3) Bennett,B. and Bloom,B.R.: Proc.Nat.Acad.Sci.,59:756, 1968

4) Pick,E.,Krejči,J.,Čech,K. and Turk,J.L.: Immunol., 17:741, 1969

5) Hashimoto,T.,Watanuki,M. and Yoshida,T.: Symp.Immunol.Biol., in press.

6) Yoshida,T. and Reisfeld,R.A.: Nature, 226:856,1970

7) in the discussion of the paper by Spector and Willoughby: Immunopathology,V:288 1967

8) Thor,D.Jureziz,R.E.,Veach,S.R.,Miller,E.and Dray,S.: Nature, 219:5155, 1968

9) Rocklin,R.E.,Meyers,O.L. and David,J.R.: J.Immunol., 104:95,1970

Fig.1
PREPARATION OF FRACTION II SHOWING MIGRATION INHIBITION AND SKIN REACTION ACTIVITIES

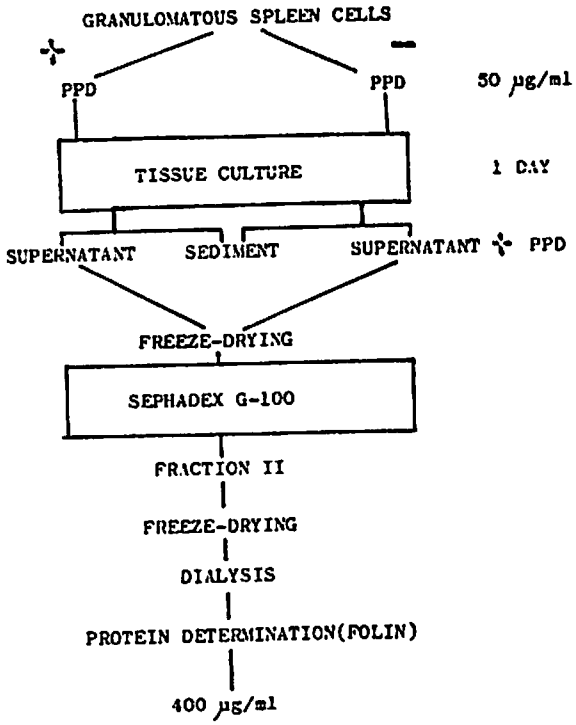


Table 1

SKIN REACTION OF VARIOUS NORMAL ANIMALS 6 HOURS AFTER INTRADERMAL INJECTION OF 40 μg Fr.II (SEPHADEX G-100) FROM GUINEA PIGS

NORMAL ANIMAL	NO. OF ANIMALS	Fr.II (%)	Fr.II (mm)
			mm
GUINEA PIG	7	17.7 ± 1.31	6.0 ± 1.59
RABBIT	6	15.8 ± 3.84	4.8 ± 2.80
RAT	6	12.1 ± 1.10	1.1 ± 1.68
MOUSE	9	0	0

Table 2. Species-specificity of MIF obtained from sensitized lymphocyte culture of guinea pigs

Source of i.p. exudate cells	% migration of macrophages in medium containing ,	
	crude supernatant	Sephadex Fr.II
guinea pigs	49.1 %	39.3 %
rats	91.3	93.7
mice	116.6	94.1

Fig.2 BIOLOGICAL ACTIVITIES OF SEPHADEX G-100 FRACTIONS

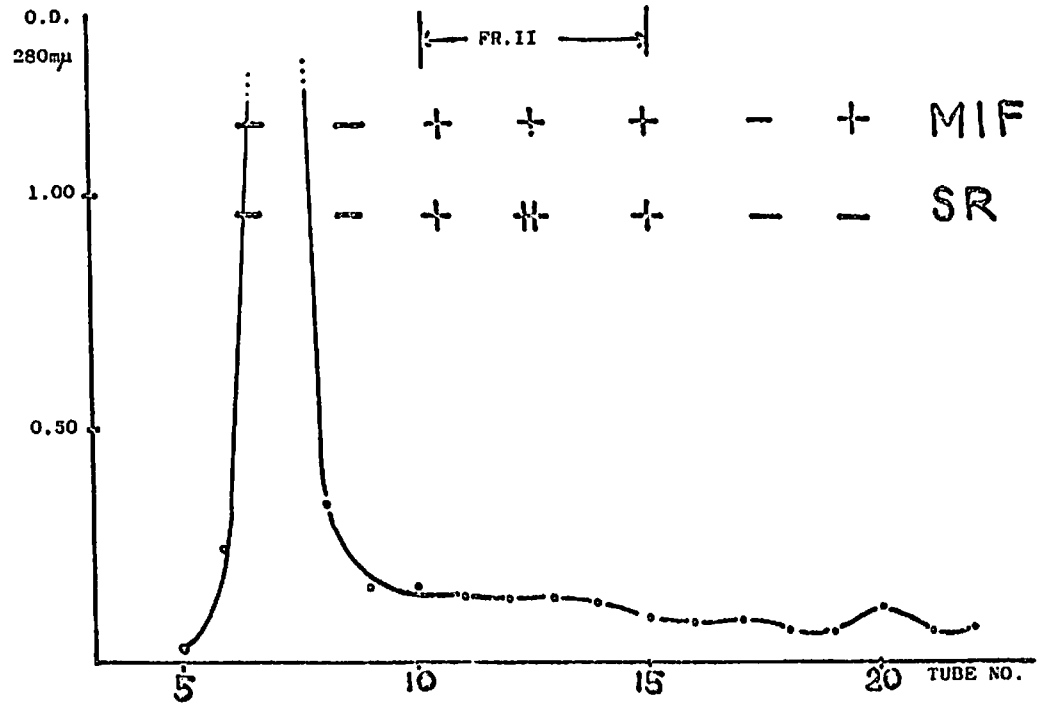


Fig.3 COURSE OF SKIN REACTION PRODUCED BY INTRADERMAL INJECTION OF 40 μg OF Fr.II FROM SENSITIZED GUINEA PIGS

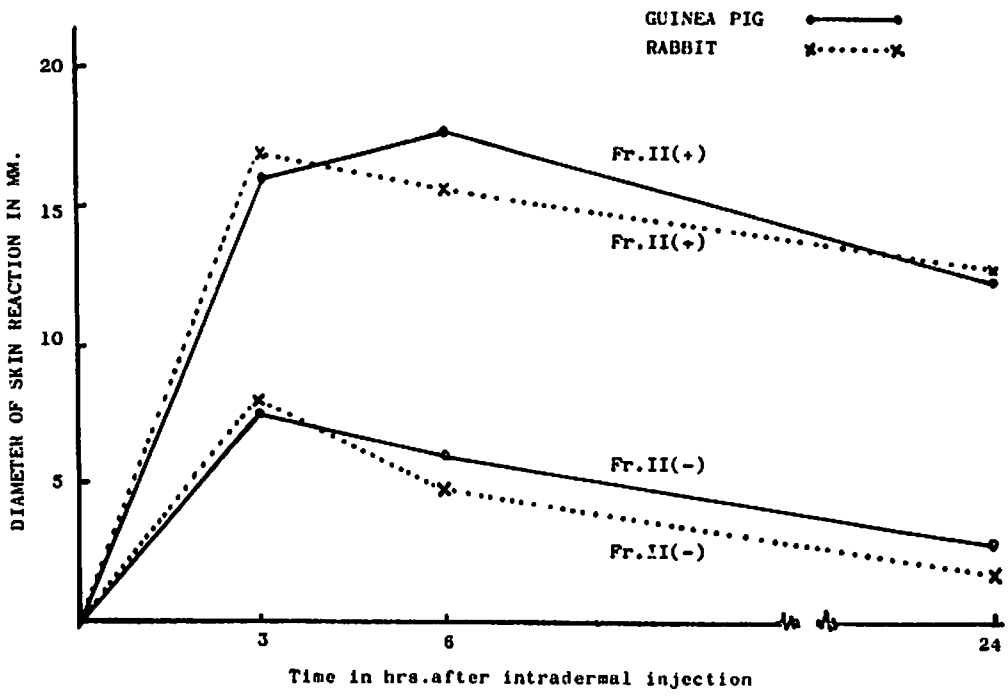
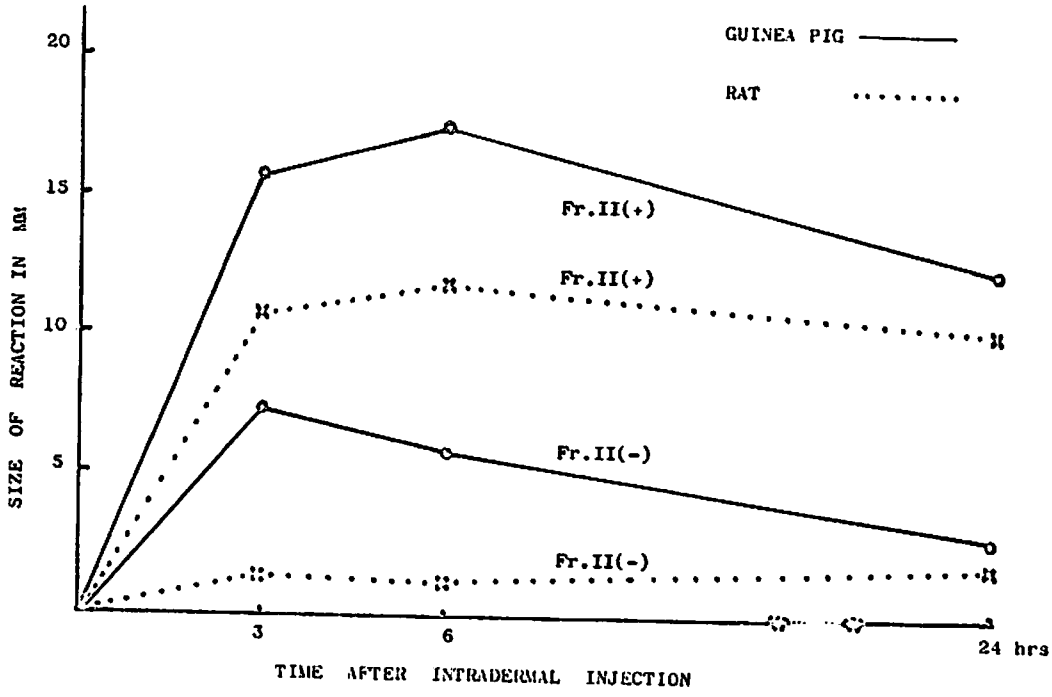


Fig.4 COURSE OF SKIN REACTION PRODUCED BY INTRADERMAL INJECTION OF 40 μg Fr.II FROM SENSITIZED GUINEA PIGS



膵癌特異抗原

北川正保（阪大・医・癌研）

Tumour specific antigens. MASAYASU KITAGAWA (Institute for Cancer Research, Osaka University)

感作腹腔内細胞による腫瘍移植抗原の識別とその変化

石館 基（癌研）

Antigenic Specificity of Hepatoma Cell Lines Recognized by Sensitized Peritoneal Lymphoid Cells.

MOTOI ISHIDATE, JR. (Cancer Institute, Tokyo)

It has been established that chemically induced tumors will have different antigenic specificity even when they have been induced by the same carcinogen in the same organ of the same individual.[1,2,3] Our study extends this finding by demonstrating that individual tumor cell line derived from different hepatoma nodules which were induced by 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) in the same liver of a single female Donryu rat, will each have a distinct antigenic specificity.

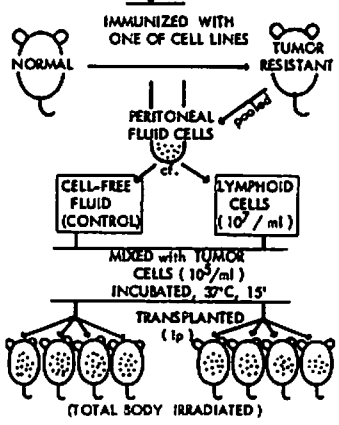
Four different cell lines of ascites hepatoma, AH-64A, AH-64B, AH-64C and AH-64D, have been established by ascitic conversion of 7 different hepatoma nodules, all of which had originated in the liver of an individual rat on the 100th day after the 3'-Me-DAB feeding for 5 months. From the AH-64C cell line, additional 4 clonal sublines, 64C-1, 64C-2, 64C-3 and 64C-4, were isolated by a technique of single cell transplantation. They have been maintained by intraperitoneal passages in female Donryu rats that have had their genetical homogeneity checked by intrastrain skin grafting.[4] Transplantability or mean survival time of the hosts when they were transplanted intraperitoneally as well as cytological characteristics such as chromosome constitution or general ascitic feature, are all individually distinct as shown in Table 1.[5]

Table 1

Hepatoma cell line	Transfer generation	Tumor take (%)	Mean survival (days)	Modal no. of chromosomes	Islands in tumor ascites (%)
AH-64A	140	98.8	16.5	66	0.9
AH-64B	140	97.5	16.5	71	83.2
AH-64C	160	99.7	10.7	81	5.2
AH-64D	150	100.0	11.9	72	7.0
64C-1	60	100.0	16.1	83	7.0
64C-2	70	99.3	9.8	75	1.2
64C-3	60	100.0	16.4	84	6.1
64C-4	60	100.0	18.2	79	14.9

In order to compare the differences in their antigenic specificity, female Donryu rats were immunized with each of these cell lines by subcutaneous inoculation of 10^7 cells which were previously attenuated with nitrogen mustard (50μg/ml) at 37℃ for 30min. When solid tumors were developed they were removed surgically. After the repeated immunizations, acquired resistance of the hosts to the corresponding tumor cell line was confirmed by further intraperitoneal inoculations of 10^5, 10^6 and 10^7 viable cells of the same cell line at 2 weeks interval. Sensitized peritoneal lymphoid cells were then pooled from the resistant animals and separated by centrifugation into cell-free fluid and lymphoid cell fractions. The cell fraction was washed with glucosol (Na HCO_3-free Tyrode) solution and adjusted to a suspension of 10^7 lymphoid cells/ml. Lymphoid cells from normal intact rats or from rats previously sensitized with normal liver cells were also prepared to serve as controls. An equal volume of a viable tumor cell suspension containing 10^5 cells/ml of each tumor cell line either corresponding to or other cell line used for immunization, was added to the separate fractions, and incubated at 37℃ for 15 min. The ratio of the number of lymphoid cells and the number of tumor cells was made constant in every case at 100 : 1. After incubation, 1ml of the mixed cell suspension was inoculated intraperitoneally into 80~100g female rats (10~15 rats in a group), that were previously irradiated with 400 rad total body X-ray. (Fig. 1)

Fig. 1



Tumor cell proliferation was then checked weekly on the smear preparations and the survival time of the hosts were recorded until 40 days after the inoculation. This method was called "Cross-Contact Transplantation Test".

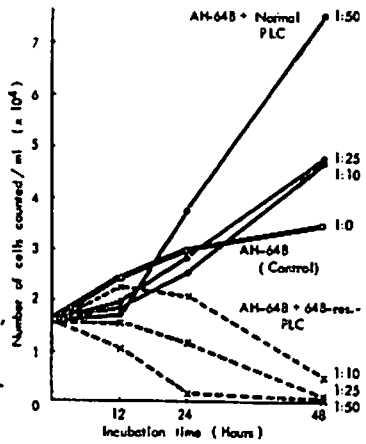
Following results were obtained from the present experiments.

1. In order to obtain tumor resistant animals, animals were immunized with tumor cells which had been attenuated with nitrogen mustard or X-irradiation and/or surgical removal of viable solid tumor mass. It was concluded that induction or acquisition of the resistance to a particular cell line was due to more antigenic property of the cell line rather than to the procedures applied for immunization. AH-64B and 64C-2 cells showed relatively high in the grade of antigenic strength when compared with other cell lines.

2. Percentages of incidence of the lymphocytes in sensitized peritoneal lymphoid cells increased following repeated immunizations. When the sensitized lymphoid cells (PLC) were mixed with tumor cells and cultured in vitro for 3 days, they showed a marked inhibitory effect on tumor cell proliferation. Fig. 2 shows specific inhibitory effect of the AH-64B-PLC on the AH-64B cells at different mixed ratio. In this figure enhancing effect of normal rat PLC on the tumor cells was unexpected.

A similar result was obtained in vivo, and increasing number of the PLC inoculated, the more prolongation of survival time of hosts was observed, while peritoneal cell-free fluid or antiserum obtained from the resistant rats showed no effect.

Fig. 2



3. By the cross-contact transplantation test (CCT-test) between tumor cell lines derived from separate hepatoma nodules, it was concluded that the inhibitory effect of PLC was specific to the corresponding tumor cell line, so that AH-64A, AH-64B, AH-64C and AH-64D-inoculated hosts showed 87, more than 100, 74 and 23% increase in 50 percent survival rate, when the AH-64A, AH-64B, AH-64C and AH-64D-sensitized PLC were inoculated, respectively. (Table 2) [6]

4. On the other hand, the CCT-test carried out between clonal sublines originating from a single hepatoma nodule, showed different results. For an example, 64C-2-PLC showed a marked prolongation effect on the hosts inoculated with not only 64C-2 cells but also other sublines. The 64C-2 cells, however, seemed to be comparatively sensitive to any type of sensitized PLC. In addition, the PLC sensitized with mother cell line, AH-64C, showed a cross reaction on either 64C-2 or 64C-4 subline cells. (Table 3)

Table 2

Cell line used for immunization	No. of rats	Percentage increase of host survival *			
		AH-64A	AH-64B	AH-64C	AH-64D
AH-64A	66	86.7	42.8	5.3	10.0
AH-64B	78	0.0	>100.0	5.9	0.0
AH-64C	66	22.7	87.5	73.5	18.2
AH-64D	55	5.6	66.8	40.0	23.0
Normal liver	45	0.0	74.0	11.8	0.0

Table 3

Cell line used for immunization	No. of rats	Percentage increase of host survival			
		64C-1	64C-2	64C-3	64C-4
64C-1	30	9.5	>100.0	37.5	40.0
64C-2	28	63.1	>100.0	46.6	100.0
64C-3	32	6.3	33.4	20.0	45.4
64C-4	20	14.3	>100.0	8.4	7.7
AH-64C	30	11.0	53.3	25.2	60.5

5. Normal PLC or the PLC sensitized with normal female rat liver cells did not show any significant prolongation of host survival. Lymphocytes obtained from the spleen or lymph nodes of the resistant animals showed less effect than sensitized PLC on survival prolongation of hosts transplanted with any type of cell line.

6. Variant sublines which showed different antigenic specificity from their mother cell line, AH-64B, were isolated when the AH-64B cells were challenged into resistant animals. Such variants could be also induced when the AH-64B cells were inoculated together with AH-64B-sensitized PLC into intact animals. (Table 4 and 5)

Table 4

Immunization (Times)	Immunized rats / Rats used	Tumor take in immunized rats (%)*	Incidence of "Variant" (%)
1	10/26	100	10
2	7/22	100	28
3	15/53	75	10
4	10/26	40	100
5	13/48	31	100
6	6/22	50	100
7	13/27	38	100
8	8/28	0	-
9	7/28	28	50
10	4/28	50	100

* AH-64B cells (10^6/ml) were challenged intraperitoneally 2~4 weeks after the last immunization.

Table 5

(10 rats in a group)

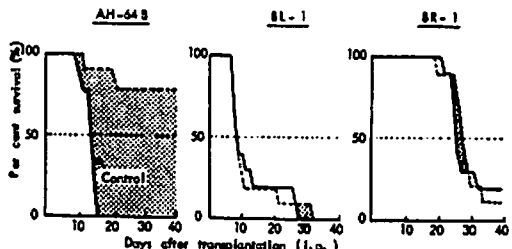
Mixed ratio PLC/Tumor	Tumor take (%)	Mean survival (Day)	Incidence of "Variant" (%)
Control	100	17.5	0
2 / 1	100	18.5	0
8 / 1	100	18.5	0
40 / 1	100	35.0	50.0 *
200 / 1	80	40.0	87.5 **

* 8L-6 ** 8L-2, 3, 4, 5

A CCT test carried out between the mother and its variant sublines, BL-1 which was isolated when contacted with AH-64B-sensitized PLC, and BR-1 which was derived from the AH-64B-resistant animal, is shown in Fig. 3. The AH-64B-PLC showed a marked inhibitory effect only on the AH-64B cells, while no effect on the other two sublines.

Similarly, by another set of CCT tests it was suggested that BL-1-sensitized PLC inhibited only the BL-1 cells, but neither AH-64B nor another variant, BR-1 cells.

Fig. 3



* Cells were transfered into the hosts previously irradiated with 400rad X-ray.

7. Cytological characteristics such as ascitic feature or chromosome constitution as well as biological nature such as malignancy in host animals or sensitivity to nitrogen mustard, were individually distinct among different sublines. General findings so far as we observed, were as follows: a) Decrease in their modal chromosome numbers. b) Deletion of the capacity to form so called hepatic islands which characterized epitherial cell origin of the AH-64B cell line.

From these results, it was suggested that rat hepatomata induced by 3'-Me-DAB may have tumor-specific transplantation antigens which can not be detected in the normal liver tissue of isologous animals, and that antigenic specificity of each cell line derived from different hepatoma nodules was individually distinct, so that the PLC sensitized with cells of individual cell line could recognize the difference and inhibit tumor cell proliferation only of the corresponding cell line used for immunization. The possible difference in antigenic specificity of separate hepatoma nodules may support strongly the concept of "multicentric origin" of chemical induced tumors from cytological point of view. [7]

Cross reaction of the sensitized lymphoid cells between different clonal sublines of AH-64C, which was derived from a single hepatoma nodule, indicated that a common antigen might be shared by these sublines. This may support our previous finding that the original AH-64C cell line has shown karyologically a mosaic cell population throughout serial passages by Donryu rats for more than 100 generations[8], since if any difference in antigenic specificity may exist among cells in the same cell population, a cell line which may be low antigenic but with an antigenic specificity, will proliferate selectively in hosts during the serial passages. One of unexpected results was the increase of survival time in the hosts inoculated with PLC sensitized to an unrelated cell line, rather than those sensitized with the same cell line used for immunization. This indicates that there are possible differences among sublines in their susceptibility to react with the sensitized PLC.

Isolation of the variant sublines from AH-64B was rather accidental. The first variant was detected in an AH-64B-resistant host 4 weeks after the last challenge of 10^6 AH-64B cells. The variant showed single cell type of ascites feature which was quite different from "island" type of the AH-64B. As similar variant sublines appeared only when the AH-64B cells were contacted specifically with AH-64B-sensitized PLC, while they could not be induced by either normal PLC or the PLC which were sensitized with any other tumor cell lines, and or antiserum from the resistant animals. In addition, such a single cell type of sublines could not be isolated by single cell transplantation from the original mother cell population. From these findings it was concluded that the variants were derived from a certain number of mother cells which acquired new characteristics on their cell surface under some specific immunological condition. Possible modification of their antigenic specificity as well as cytological characteristics in the hosts resistant to tumor cell transplantation, may support a possibility that tumor cells at metastatic region or those in recurrent site would have different antigenic specificity when compared with that of the primary tumor. [9, 10]

References : 1) Klein, G, Sjögren, H. O., Klein, E. & Helström, K. E. Cancer Res., 20, 1561 (1960). 2) Globerson, A. & Feldman, M., J. Nat. Cancer Inst., 32, 1229 (1964). 3) Rosenau, W. & Morton, D. L., J. Nat. Cancer Inst., 36, 825 (1966). 4) Ishidate, M. Jr., Gann, 58, 5 (1967). 5) Idem, Gann, 59, 341 (1968). 6) Idem, Nature, 215, 784 (1967). 7) Yoshida, T., Deut. Med. Wochschr., 88, 2229 (1963). 8) Ishidate, M., Jr., Abstr., 12th Internat. Cong. of Genetics (1968). 9) Hamond, W. G., Abstr., 59th Ann. Meeting, Proc. Am. Assoc. for Cancer Res. (1970). 10) Kim, U., Science, 167, 72 (1970).

マウス乳癌の腫瘍特異抗原の精製と化学分析

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Chemical analysis of Further Purified Tumor Specific Antigens of Mouse Mammary Tumors.

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ABSTRACT

The solubilized tumor specific transplantation antigen of mouse mammary tumor MM102 (Irie et al., 1969) was further purified by 75 % ethanol (EtOH Ag fraction) and its biological activity and chemical composition were analysed. Recovery of the antigenic activity tested by immune adherence inhibition test was 25 %, and the antigenic activity was found to be purified by 72 folds on dry weight basis (TABLE I). This fraction inhibited the cytotoxic activity of the tumor specific antiserum (RMS), and induced heightened resistance against MM102 in the syngeneic C3H/He mice. By these experiments the fraction was confirmed to contain a transplantation antigen. Chemical analysis of the lyophilized EtOH Ag fraction showed that it was composed of 11.7 % protein, 83.3 % lipid, 0.35 % sugar, and 3.9 % RNA (TABLE II). Lipid analysis showed that the ratio between phospholipid and neutral lipid in lipid moiety was 44:56 and the content of sphingomyelin in phospholipid was 19.3 %.

The localization of the antigen on the mitochondrial and plasma membrane in MM102 cells was demonstrated by electronmicroscopic analysis using RMS conjugated with peroxidase. From lipid analysis, however, it was suggested that the antigenic material might be extracted only from the plasma membrane.

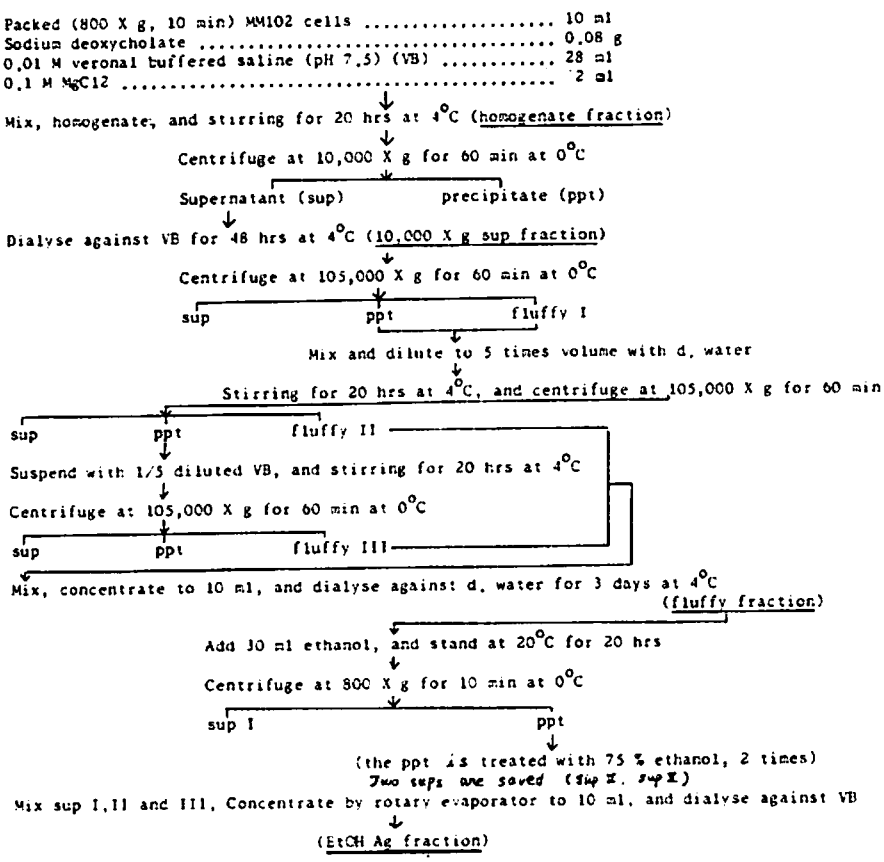
None of chloroform-methanol (2:1, V/V), ethanol-ether (3:1, V/V) and aceton (90 %) extracts from MM102 showed any antigenic activity. The antigenicity of EtOH Ag fraction was found to be lost through the separation of its free lipid or by the treatment with phospholipase A or C on it. Lipids in the EtOH Ag fraction might be expected to be useful for the formation and maintenance of the antigenicity as observed in the studies of enzyme, Rh antigen and Semliki Forest Virus.

マウス乳癌の腫瘍特異抗原の証明は，その大部分が同系マウスを用いた移植免疫によってなされており，その抗原の物質的な解明に関しては，特異抗体が得られないため余り進められていない．我々はマウスの自然発生乳癌を腹水化した継代移植癌を用いることによって血清中に特異抗体を証明することに成功し，更にその抗体を用いて抗原の精製及び化学分析を行ったので報告する．

腫瘍は同系C3H/Heマウスの自然発生乳癌を腹水型にしたMM102細胞で

ある。特異抗体は，Sodium Deoxycholate を用い MM102 から抽出した抗原分画を，同系 C3H/He マウスに免疫して得た高力価の血清抗体（RMS）である。精製過程における各分画の抗原活性は RMS を用いた Immune Adherence 阻止実験によって積べた。現在進められている段階までの精製法を Figure 1 に示した。

FIGURE 1. PURIFICATION PROCEDURE OF THE TUMOR SPECIFIC ANTIGEN OF MOUSE MAMMARY TUMOR MM102.



精製過程における各分画の抗原活性の回収率と乾燥重量を TABLE I に示した。EtOH 抗原分画の，homogenate 分画の活性からみた回収率は 25 % である。10,000 x g 沈殿および 105,000 x g 沈殿分画にも抗原活性が検出されることや，エタノール処理での失活などが回収に大きく影響したと思われる。乾燥重量当りにすると，EtOH 抗原分画は 22 倍精製されたことになる。

これらの分画の化学分析は凍結乾燥した材料について行った。結果は TABLE II に示してある。Homogenate，10,000x g，fluffy 分画については，それらの化学組成は極んど同じで，蛋白質 60～70 %，脂質 10～20 %，糖 1 % RNA 10～20 %

である。Fluffy 分画をエタノールで処理することにより大量の蛋白質と RNA が除かれ，脂質は 50% が EtOH 抗原分画に回収されるため，蛋白質：脂質の割合が大きく逆転している。

TABLE I. PURIFICATION AND RECOVERY OF ANTIGENIC ACTIVITY DETERMINED BY 50 % ABSORPTION OF RMS[1] IN INHIBITION TEST OF INMUNE ADHERENCE.

Fractions	Dry weight[2] recovery (µg)	Relative activity	Dry weight required for 50 % absorption of RMS (µg)
Homogenate	1160	100	685
10,000 x g sup	632	61	606
Fluffy	174	33	286
Dialysed fluffy[3]	174	54	191
EtOH Ag	13.5	25	31

1 ; the tumor specific antiserum.
2 ; started from 2.2 x 10^6 MM102 cells which showed 50 % absorption of RMS.
3 ; The fluffy fraction was dialysed against deionized water before dialysis by veronal buffered saline.

TABLE II. CHEMICAL COMPOSITION OF THE DIFFERENT FRACTIONS.

Fractions	Component (%)[1] Protein	Lipid	Sugar[2]	RNA	DNA
Homogenate	60.6	16.2	1.16	13.7	8.18
10,000 x g sup	73.5	10.9	0.89	14.7	<0.13
Fluffy	67.3	10.9	1.08	20.2	<0.12
EtOH Ag	11.7	84.2	0.356	3.86	<0.099

1 ; percentage on the basis of dry weight.
2 ; sum of the hexosamine and sialic acid.

Peroxydase で処理した RMS と MM102 細胞と反応させ，電子顕微鏡による抗原の局在を観察したところ，ミトコンドリア膜とプラスマ膜に電子密度の高い染色が証明された。しかし EtOH 抗原分画の脂質分析の結果からは，(リン脂質と中性脂質の割合が 44：56，リン脂質中のスフィンゴミエリンが 19.3% であることなど) この分画が細胞膜由来のものであることが示されている。

EtOH 抗原分画について 75% エタノールを展開溶媒にして Thin layer chromatography で脂質分析したところ，遊離の脂質が大量に存在することが証明されたので，これらを除いた残りの抗原活性を調べたら，殆んど活性が失なわれていた。Phospholipase A やこの処理によって活性がなくなることから脂質が重要な役割を担っているように思われるが，クロロホルム－メタノール (2：1)，エタノール－エーテル (3：1)，アセトン (90%) などによって全脂質を抽出しても，それに匹敵する活性も認められなかったことなどから，この

抗原決定基は脂質自身ではないが脂質の関与が必要であろうことが示唆された。酵素活性や、防疫原などで証明されているごとく、これら脂質は、マウス乳癌の腹痛特異抗原の形成或は維持に役立っているのかもしれない。

Xenogenization of rat cells by infection with Friend virus

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Friend virus is highly infectious to the mouse, but it is not infectious to the rat. It is known that the Friend virus is a membrane producing leukemogenic virus, so that it is interesting to see what happens if the rat cell was infected with Friend virus under certain specified conditions. This is because the new antigen produced in the rat cell by infecting them with Friend virus may be originally "foreign" to the host. In other words, attempts were made to analyse the relations between the Friend virus-induced new antigen and the host.

Wistar King Aptekman/Mk (WKA/Mk) rats injected intraperitoneally and subcutaneously with 10^5 ID50/ml Friend virus at birth matured well as noninjected normal rats. However, as it was reported previously[1], approximately 200 days after the Friend virus inoculation rats develop neoplastic enlargement of the spleen and thymus. The histopathology of most of the neoplasms was lymphosarcoma.

The most characteristic finding in these neoplasms from the rat injected with Friend virus at birth was observed at the transplantation of the tumor.

A) Although Friend virus-induced tumors (RFT) show the histology of a typical malignant neoplasm and WKA/Mk rats are inbred, the RFT-tumors either will not grow in the isologous normal adult rat, or even if they grow initially then regress later in all cases (Table 1).

B) In order to obtain the RFT-tumors proliferating in vivo, Friend virus-conditioned rats 1-2 months old after the Friend virus injection at birth were used. This is because the RFT-tumors grow well in such Friend virus-conditioned rats with the metastatic growth of the tumor and kill the host as usual experimental tumors do.

Table 1. Growth of Friend virus-induced tumors in rats(RFT)

	In normal rats		In Friend virus-conditioned rats	
WPT-1	3/38	11%	128/129	99%
-2	0/53*	0	113/113	100
-3	0/26	0	58/58	100
-4	0/9	0	25/25	100
-5	-		9/12	75
-6	0/4	0	9/9	100
-7	0/3	0	7/13	54
-8	0/5	0	11/11	100

* Spontaneous regression

As for the mechanism of the above curious transplantation result of the Friend virus-induced tumors in rats, the following information is available.

A) The difficulty of the RFT growth in the isologous normal rat may be due to the fact that the membrane antigen produced on the cell surface of the RFT is caused by the infection of Friend virus which is poorly infectious to the rat, so that the membrane antigen of the RFT may be recognized as foreign and be rejected by the host. A similar curious transplantation result was also observed in tumors of the rat which had been induced by chemical carcinogens or developed spontaneously. By artificially infecting them with the Friend virus the tumors were converted to tumors incapable of growing in isologous normal adult rats[2] (Table 2). Cytotoxic test or membrane fluorescent technique also proved that the above explanation may be appropriate[3),4)].

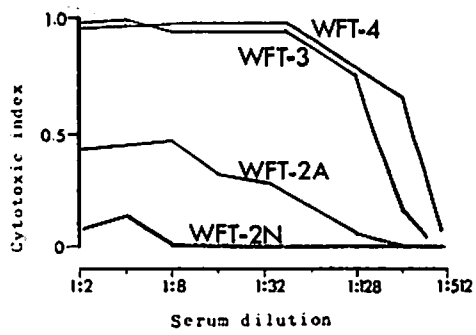
B) The reason that the RFT-tumors grow in Friend virus-conditioned rats may be due to the fact that Friend virus-conditioned rats are Friend virus tolerant and also tolerant to the Friend virus-specific membrane antigen. Although the virus neutralizing- and cytotoxic antibodies were observed at a high level in the Friend virus immunized adult rat, no such antibodies were observed in the serum of the Friend virus-conditioned rat. However, cellular responses in the Friend virus-conditioned rat seem to be normal, because the allogeneic skin- and tumor transplantation was rejected, as in normal rats. The RFT-tumor grows not only in the above specific tolerant rat, but also it grows in the rat heavily irradiated or suppressed immunologically.

Table 2. Lethal growth of Friend virus-infected tumor cells in normal rats.

Tumor	Friend virus-infected cells	Non-infected cells
WST-5	0/26	19/21
DLT	0/28	26/26
KMT-68	0/24	26/26
AH109A	2/8	16/16
Takeda sarcoma	6/18	14/18
	0/7	7/7
Total	8/111 (7.2%)	108/115 (94.7%)

Thereafter, a variant line (WFT-2N) was obtained from one of the RFT-tumors (WFT-2A)[5]. The characteristics of the WFT-2N is that this will grow in the normal adult rat and kill the host. Only this is different from other conventional RFT-tumors. Cytotoxic test is inversely related to the transplantation result; anti-serum in rats immunized with Friend virus or WFT-3, one of the ordinary type of RFT-tumors, did highly react in the cytotoxic test with WFT-3, WFT-4 and other RFT, and intermediately reacted with WFT-2A, but did not react with

Fig. 1 Cytotoxic sensitivity of anti-WFT-3 rat serum to various lines of the RFT.



WFT-2N (Fig. 1). These correspond to the above mentioned transplantation results in which WFT-2N do grow as usual tumors, WFT-2A grows well for a while and then regresses eventually. Other remaining lines of RFT-tumors do not grow at all in the normal rat. WFT-2N, after the treatment with neuraminidase, begins to react with the antiserum in the cytotoxic test.

Electric properties of the various lines of RFT-tumors were investigated[6]. As result, it was confirmed that except for the WFT-2N electric properties of RFT-tumor cells were nearly the same as those in normal lymphocytes of the rat (Table 3.). Neoplastic cells having such electric properties of the normal cells had never been reported, and further studies may be required for analizing the mechanism of such curious properties of the membranous surface of the cell.

Table 3. Electriphoretic mobilities of viable cells

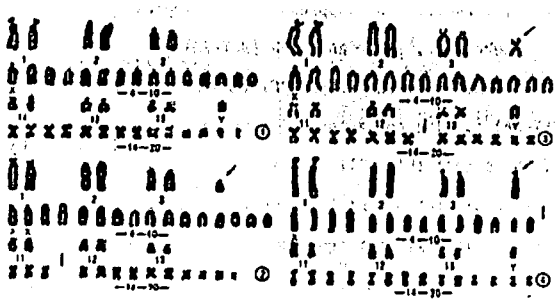
Yoshida sarcoma	-1.310	± 0.100
SPAT-1*	-1.257	± 0.043
WFT-2N	-0.960	± 0.069
WFT-2A	-0.938	± 0.088
WFT-3	-0.897	± 0.053
Normal rat lymphocytes	-0.911	± 0.046

/sec/V/cm

* Friend virus-induced tumor of mice

Another interesting result was found in the chromosomes of the RFT-tumor cells, in which the normal pattern of the chromosome was observed in most of the lines of RFT-tumors as observed in the normal cells of the rat. Other remaining lines of the RFT-tumor indicated a very slight change of the chromosome (Fig. 2)[7].

Fig. 2. Representative stemline karyotypes of the ① WFT-3, ② WFT-4, ③ WFT-2A, and ④ WFT-2N



In summary, Friend virus-induced tumors in rats (RFT), except for the variant, seemed to be an unreal malignant neoplasm because of their curious transplantation results, cytotoxic tests, electric properties and chromosomes of the cell. However, the evidence that the RFT-tumors are the real neoplasm may be given in their histology and invasive growth of the tumor in Friend virus-tolerant rats. Such curious biological behaviour of the RFT-tumors may be due to the fact that the tumors originated from rats injected with the Friend virus, which is originally foreign to the rat and naturally a membrane producing virus. Friend virus-induced tumors in mice have never indicated such curious behaviour of the tumor as observed in the rat. In other words, it seems that the above results may be expressed only when the rat was injected with Friend virus. We designate, therefore, the RFT-tumors as one of the "noncancerous cancers"[8].

As it will be reported eleswhere[9], when the newborn rat is injected with a small amount of the

Friend virus, which is not adequate to result in the immunological to tolerance of the host, the rat will soon die of the runting syndrome. This may be caused by some immunological disorders, in which the Friend virus-infected lymphocytes may be inhibited by noninfected normal lymphocytes of the host. We refered to the above runting syndrome resulting in death of the host as "xenogenization disease". It has been also mentioned that both the rat tumors developed spontaneously or induced by chemical carcinogens without any connection to the virus were converted to tumors acquiring some foreign antigen on the cell surface by artificial infection with Friend virus and were followed by the regression of the tumor. This is designated as "artificial xenogenization of the tumor"[2),10),11)]. In any event, it can be said that the above curious biological behaviours represented by the Friend virus induced-tumors (RFT) may be obtained from the "hybridization" of the cell and the virus, both recovered from animals of different species.

References:

1) Kobayashi, H., Hosokawa, M., Takeichi, N., Sendo, F. and Kodama, T.: Transplantable Friend virus-induced tumors in rats. Cancer Res., 29: 1385-1392, 1969.

2) Kobayashi, H., Sendo, F., Shirai, T., Kaji, H., Kodama, T. and Saito, H.: Modification in growth of transplantable rat tumors exposed to Friend virus. J. Nat. Cancer Inst., 42: 413-419, 1969.

3) Shirai, T., Kaji, H., Sendo, F., Kobayashi, H.: Cell surface antigens detectable by cytototxic test on Friend virus-induced and Friend virus-infected tumors in the rat. J. Nat. Cancer Inst., accepted.

4) Saito, H., Takeichi, N., Sendo, F., Kaji, H., Kobayashi, H.: Immunofluorescent studies on the Friend virus-infected tumor cells in rats. Gann, submitted

5) Kobayashi, H., Shirai, T., Takeichi, T., Hosokawa, M., Saito, H., Sendo, F. and Kodama, T.: Antigenic variant (WFT-2N) of a transplantable rat tumor induced by Friend virus. European Journal of Clincial and Biological Research, 15: 462-468, 1970.

6) Kojima, K., Takeichi, N., Kobayashi, H. and Maekawa, A.: Electrokinetic charge of transplantable Friend virus-induced tumors in rats. Nagoya Medical Journal, 16: 7-13, 1970.

7) Sasaki, M., Mori, M., Kobayashi, H., Takeichi, N.: Cytologic studies of tumors, L2. Persistence of diploid stemlines in Friend virus induced ascites tumors of rats. Proc. Japan Acad., 46: 1970 (in press)

8) To be published

9) Takeichi, N., Kuzumaki, N., Kobayashi, H.: Runting syndrome in rats inoculated with Friend virus. Gann, submitted

10) Kobayasni, H., Kodama, T., Shirai, T., Kaji, H., Hosokawa, M., Sendo, F., Saito, H. and Takeichi, N.: Artificial regression of rat tumors infected with Friend virus (xenogenization)---An effect produced by acquired antigen. Hokkaido Med. J., 44: 134-135, 1969.

11) Sendo, F., Kaji, H., Saito, H. and Kobayashi, H.: Antigenic modification of rat tumor cells artificially infected with Friend virus in the primary autochthonous host. Gann, 61: 223-226, 1970.

CELL MEDIATED IMMUNITY IN CANCER PATIENTS

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This subject has two separable but interrelated parts. The first concerns the adequacy of immune mechanisms in patients with cancer. The second concerns the existence and efficacy of anti-cancer defenses. Our interest is not limited to cell-mediated reactions, but these are of particular interest because there is a characteristic depression of cell-mediated immune reactions in patients who have advanced cancer, and because most students of tumor immunology agree that immunologic defenses against cancer are principally of the cell-mediated type.

I will not attempt to cover the second part of this subject, but the following papers by Dr. I. Hellstrom and Dr. K. Kikuchi will be concerned with cell-mediated immune reactions against autochthonous cancer in humans and experimental animals.

I will attempt to summarize what is now known concerning the general immunologic capability of cancer patients. I will emphasize cellular mechanisms, as measured by primary and secondary delayed hypersensitivity reactions, rejection of first and second set homografts, mobilization of macrophages, and phagocytic activity of the reticuloendothelial system, but will also touch upon humoral mechanisms including production of 7S and 19S serum antibodies, serum complement components, and serum "properdin" activity.

In patients with advanced cancer there is frequently a deficiency in delayed hypersensitivity reactions, and a delay in rejection of homografts of normal or neoplastic cells. There is also evidence that mobilization of macrophages is impaired, but phagocytic activity as measured by up-take of colloidal particles from the blood is normal or even faster than normal. There is no evidence of impaired production of antibodies of the IgM (19S) or IgG (7S) type, but the representation of other types of immunoglobulins in the antibody response in cancer patients has not yet been investigated. In general, serum complement activity and the several components of the complement system are present at high normal levels in cancer patients. The "properdin" activity of serum is markedly depressed in about half of all cancer patients. In many patients this deficiency appears to be due to inhibitors of the serologic reaction rather than an actual lack of "properdin". The titration of properdin however, probably measures a variety of antibody-like factors, so the precise nature of the deficiency and its immunologic significance are entirely unknown.

This pattern of immunologic deficiency occurred in patients with epidermoid and adenocarcinomas and sarcomas of diverse origins. In patients with primary neoplasms of lymphocytes and plasma cells (lymphosarcoma, lymphocytic leukemia, multiple myeloma) production of serum antibodies may also be impaired, in addition to the impairment of cell-mediated reactions. Since these are neoplasms of the cells which actually produce serum antibodies, this impairment is presumably attributable directly to the disease process, but the available data do not actually confirm nor refute this logical assumption, since most if not all patients with these diagnoses whose immune responses have been studied have also received immunosuppressive types of anti-cancer therapy (radiotherapy or chemotherapy). However, delayed homograft rejection and impaired DNFB response occurred in many carcinoma patients who had not received immunosuppressive treatment, and these deficients did not occur in severely debilitated patients who had non-neoplastic diseases, so this deficiency of cell-mediated immunoligic reactions is apparently related to cancer per se. Patients with less advanced cancer who were in good physical condition showed no immunologic inadequacy that was detectable with these crude and non-quantitative methods, so it appears that the characteristic immunologic deficiency of cancer increases in severity as a concomitant of progressive tumor growth, but we cannot dismiss the possibilities that the deficiency actually exists in lesser degree before the onset of neoplasia, or early in the course of the disease, and that it may contribute to the pathogenesis of the disease.

Cell-mediated immunity against tumors

Ingegerd Hellström (Department of Microbiology, University of Washington)

Rejection of allografted tissues is mediated primarily by immunologically competent cells. Such cells also play the primary role in the rejection of tumors which contain foreign specific antigens.

Many techniques have been introduced by which cell-mediated immunity can be detected in vitro. We have developed and utilized a so-called colony inhibition assay, by which tumor cells are plated onto plastic petri dishes and the following day, when they have attached to the surface of the dishes, superseeded with a suspension of lymphocytes immune to the antigens of the neoplastic target cells (lymphocytes immune to some other, non-crossreacting antigens being used in the controls). The ability of the specifically immune, as compared to control, lymphocytes to reduce colony formation in vitro of the plated target cells is measured. Lately, we have also utilized a cytotoxic test, which is carried out on Falcon microtest plates, and in which we count the number of surviving target cells rather than colonies formed.

It has been possible to show that lymphocytes from animals specifically immunized to antigenic tumor cells, as well as from animals whose own, autochthonous or transplanted syngeneic tumors have been removed, are inhibitory (cytotoxic) to cultivated neoplastic cells of the respective types. Such an inhibitory (cytotoxic effect can also be seen with lymphocytes from animals carrying progressively growing neoplasms. Many different animal systems have been studied by now and give very similar types of data: polyoma virus induced tumors of mice and rats, methylcholanthrene induced sarcomas of mice and rats, adeno 12 virus induced tumors, spontaneous mouse mammary carcinomas, Shope virus induced papillomas of rabbits, Moloney virus induced sarcomas of mice.

A major part of our effort has gone into trying to find out why tumors can grow progressively in vivo in spite of the fact that their cells can be recognized as antigenically foreign in vitro and there killed by the tumor-bearing individuals' lymphocytes. The most likely explanation of this situation (also other mechanism are likely to play a role) comes from the demonstration that sera from animals with progressively growing tumors contain factors (presumably enhancing 7s antibodies), which can in a specific way protect the same animals' tumors from destruction in vitro by their immune lymphocytes. Such enhancing or blocking factors have been demonstrated in all animal systems so far investigated: Shope virus papillomas of rabbits (in the persistor and carcinoma rabbits, not in the regressors), Moloney virus induced sarcomas of mice (in animals with growing tumors, not in those whose tumors have regressed), methylcholanthrene induced sarcomas in mice and rats, polyoma virus induced sarcomas in mice and rats (Sjögren et al), spontaneous mouse mammary carcinomas (Heppner). The blocking serum activity is formed in animals splenectomized before tumor transplantation (or induction). The blocking molecules can be removed by absorbing sera with the respective target cells.

On the basis of our experience from animal experiments, we have conducted searches for a cell-mediated tumor immunity in the human systems and for a blocking effect of serum from patients with growing tumors. These studies have shown that a cell-mediated immunity can indeed, be detected against human neoplasms and that patients with progressively growing tumors, but not the cured ones, have blocking serum factors, which are most likely 7s immunoglobulins (or, possibly, antigen-antibody complexes). One of the most interesting findings evolving from these studies is that histologically similar types of tumors have cross-reacting antigens.

Thus lymphocytes from patients with one type of tumor, for example neuroblastoma, are commonly cytotoxic (inhibitory) to tumor cells of the same type from other patients as well. Other groups of neoplasms within which immunological cross-reactions have been detected include malignant melanomas, breast carcinomas, endometrial carcinomas, colon carcinomas, seminomas, ovarian carcinomas, sarcomas. No cross-reactions have been detected between tumors of different types. Normal cells from the tumor patients have not been affected by lymphocytes destroying the same patients' neoplastic cells.

We have recently obtained evidence that the tumor specific antigens of colon carcinomas, involved in the destruction of such cells by immune lymphocytes, may be of a carcinoembryonic type (analogous with --the same?) as that described by Gold and Freedman, since lymphocytes from patients with colon carcinomas inhibit colony formation of cultivated fetal gut epithelium but not of fetal kidney cells (or of cells from adult colon mucosa).

Implications of our findings with relation to tumor prophylaxis and tumor therapy will be discussed.

Cell-mediated Immunity and Allogeneic Inhibition of Tumor Bearing Hosts.

KUNZO ORITA, EIJI KONAGA, NOBUYUKI OHNISHI, NORIHIKO TERADA, YOSHIAKI KOKUMAI, SANAE TANAKA (Department of Surgery, Okayama University)

For the past several years we have been conducting studies on cancer immunity from the aspect of transplantation immunity by in vitro tissue cultures. It has been possible to demonstrate that even cancer-bearing host destined to die if left untreated shows anti-tumor activity by way of its own lymphoid system at a certain stage, but beyond a certain limit when the tumor aggravates it loses such an anti-tumor capacity. Fundamentally such a phenomenon can be observed both in animals and human beings. Namely, it has already been demonstrated that in the mixed tissue cultures of lymphocytes from a tumor-bearing host with respective cancer cells of the host, these lymphocytes aggregate on the target cancer cells and inhibit the growth of the latter ultimately leading the latter to destruction. This has been proven to be the same in the mixed cultures of such lymphocytes from a tumor-bearing host with cell lines like mouse mammary cancer cells, methylcholanthrene-induced sarcoma cells, either after homotransplantation or isotransplantation, and also in the mixed cultures of such lymphocytes with spontaneous mouse mammary cancer cells or with human cancer cells, the phenomenon is observable invariably in all the mixed cultures.

Now, looking at the anti-tumor effect of such lymph node cells of a tumor-bearing host from the relationship of cancer development to the location of the lymph nodes, it seems that once a cancer generates or is transplanted, the regional lymph nodes of the host acquire anti-tumor activity, while as the cancer grows beyond a certain biological limit the anti-tumor activity of regional lymph nodes decreases gradually, and anti-tumor activity develops in distal and distant lymph nodes, and as the cancer grows still bigger, even the anti-tumor activity of distant lymph nodes is lost. For example, in our experiments of mixed cultures of regional axillary lymph node cells, spleen cells, or distant mesenterial lymph node cells, taken out one, two and 3 weeks after homotransplantation of 500 x 10^4cells/ml Ehrlich ascites tumor cells on the back between scapulas of Cb mice, mixed with JTC-11 cells (derived from Ehrlich cancer) in the ratio of 20 : 1, we find anti-tumor effect in lymph node cells as proven by the cell counts of target cells (cancer cells) in culture. That is, by one week after transplantation regional lymph node cells show a weak anti-tumor activity but no such anti-tumor activity in the distant lymph node cells or in spleen cells, by 2 weeks a strong anti-tumor activity in regional lymph node cells and spleen cells, by 3 weeks the activity is decreased or obliterated in both of these cell groups, while a strong activity in the distant mesenterial lymph node cells. Similar findings can be obtained in the case of isotransplantation of methylcholanthrene-induced (MC) sarcoma. For this test first the MC sarcoma induced in C3H mouse is transplanted in C3H mouse subcutaneously on the back and regional axillary lymph nodes, spleen and mesenterial lymph nodes are taken out at certain intervals of week, and respective lymphocytes are added to the replicate cultured MC sarcoma cells in the ratio of 20 : 1 (lymphcytes : target sarcoma cells). In such mixed cultures, with those lymph node cells obtained one

week after the transplantation regional lymph node cells show a slight anti-tumor activity but no such activity in the distant and mesenterial lymph node cells. By 2 weeks the regional lymph node cells show the most marked anti-tumor activity followed by the spleen cells and mesenterial lymph node cells. However, by 3 or 4 weeks such activity is most marked in the distant mesenterial lymph node cells, followed by that of regional lymph node cells and the spleen cells. In our mixed culture trials with a few human cases, when each group of these regional and distant lymph node cells are mixed with autologous primary replicate cultured cancer cells, we find that 54% of lymph node cells show anti-tumor activity while 82% of distant lymph node cells. This finding seems to suggest that the cases with large tumors as observed in clinics can be considered to correspond the terminal stage in animal cancer.

On the other hand, Hellström and Möller have proposed "allogeneic inhibition" to constitute one of the surveillance mechanisms, arousing a deep interest as to its probable association with cell-mediated immunity. In our observations of normal lymphocytes treated with 1-2% phytohemagglutinin (PHA-M) for a certain period of time or normal lymphocytes left in the culture medium containing PHA, these normal lymphocytes, as if they were sensitized, aggregate, inhibit and destroy the target cells, JTC-11 cells (derived from Ehrlich ascites tumor). Judging from these phenomena of aggregation and growth inhibition, cell-mediated immunity seems to resemble closely allogeneic inhibition.

For the full understanding of the action mechanism of cancer-bearing mouse lymphocytes, which is cell-mediated immunity, it would be of significance to analyze similarity and dissimilarity of cell-mediated immunity and allogeneic inhibition. For just such a purpose we have compared the effect of regional lymph node cells from the Ehrlich tumor bearing mice on target cells, JTC-11 cells, 2 weeks after subcutaneous transplantation of 500 x 10^4 cells/ml of the tumor, with the allogeneic inhibitory activity of PHA-treated normal mouse lymphocytes on JTC-11 cells. As a result we find that those lymphocytes from cancer-bearing host specifically aggregate and inhibit only the growth of the Ehrlich cancer cells which was transplanted, but the PHA-treated normal mouse lymphocytes show not any specificity.

The lymphocytes of tumor bearing mouse invariably begin to attach uniformly around JTC-11 cells by culture hour 5-6, and by 24 hours the destruction of the JTC-11 cells can be observed. The PHA-treated normal mouse lymphocytes begin to aggregate on one side of JTC-11 cells by culture hour 2-3 and destroy the latter by culture hour 6-10.

In the presence of 25μg/ml hydrocortisone in the culture medium, apparently the aggregation of cancer-bearing mouse lymphocytes is not inhibited but their anti-growth effect is lost. In contrast, in the case of the PHA-treated normal mouse lymphocytes the presence of hydrocortisone has no effect on their aggregation and growth inhibition.

When the cancer-bearing mouse lymphocytes are treated with 10,000r x-ray prior to the culture, they lose growth inhibitory effect on JTC-11 cells, but PHA-treated normal mouse lymphocytes similarly irradiated do not lose their growth inhibitory effect.

When cancer-bearing mouse lymphocytes are washed repeatedly with 0.01% trypsin solu-

tion and then cultured with JTC-11 cells, the anti-growth effect of these lymphocytes is lost as well as PHA-treated normal mouse lymphocytes similarly treated with trypsin lose their anti-growth effect.

The supernatant fraction obtained after centrifugation (100,000g for 60 min) of homogenate of cancer-bearing mouse lymphocytes has anti-growth effect on JTC-11 cells, but with normal lymphocytes, which have acquired allogeneic inhibitory activity by being treated with 2% PHA for 12 hours, when washed thoroughly by Hank's solution and homogenated, then the homogenate centrifuged (100,000g, for 60 min), the supernatant thus obtained shows no growth inhibitory effect.

In addition, when cancer-bearing mouse lymphocytes are treated with PHA, their anti-growth effect is not accelerated. From these findings it seems that sensitized lymphocytes, without aid of PHA or heterogenous antiserum, do attach specifically to antigenic cells, inhibit the growth and destroy the antigenic cells, and this specific attachment is a real expression of cell-mediated immunity, and at the stage of subsequent cell destruction similar to allogeneic inhibition is operating. It is assumed that there is an antibody-like receptor on the surface of the sensitized lymphocyte.

In comparing the changes in the cell-mediated immunity with those of the allogeneic inhibitory activity of the host along with the development of cancer, an intimate relationship between these two phenomena seems to become clearer.

For this comparative observation, we carry out isotransplantation of MC-induced sarcoma subcutaneously on the back of a few C3H mice successively at interval of one week, making 4 groups, and regional axillary lymph nodes are taken out from each group 4 weeks after isotransplantation. Then the regional lymph node cells are mixed with each group of the primary replicate cultured MC-cells in the ratio of 20 : 1, and cutured for 24 and 48 hours to determine the activity of cell-mediated immunity of the regional lymph node cells. Simultaneously, these regional lymph node cells are added to JTC-11 cells (derived from Ehrlich cancer) in the ratio of 40 : 1 in the presence of 2% PHA and cultured to see the activity of allogeneic inhibition of these lymphocytes. As a result it is found that the anti-tumor effect on the primary culture sarcoma cells of the lymphocytes taken out one or two weeks after isotransplantation is strong, and also the allogeneic inhibitory effect on Ehrlich cancer is strong. However, by 3 or 4 weeks when cancer has progressed, both the cell-mediated immunity capacity and allogeneic inhibitory effect are decreased. It seems, therefore, the mechanism of allogeneic inhibition, which is one of the surveillance mechanisms, is deeply involved in the cell-mediated immunity.

These findings lead us to conclude that in the terminal stage of cancer the cell-mediated immunity as well as the allogeneic inhibition are lowered, making it difficult to cure cancer.

担癌宿主における cell-mediated immunity と allogeneic inhibition について
新田薫三，小長英二，大西佑行，寺田紀彦，国米欽明，田中早苗

Studies on the role of blocking serum factors (antibodies?) under some conditions not involving neoplasia

Karl Erik Hellström (Department of Pathology, School of Medicine, University of Washington)

We have during the last few years employed a so-called colony inhibition assay to search for cell-mediated immunity against tumor specific antigens. One of the most striking findings from studies carried out with that assay was that lymphocytes from tumor-bearing animals and human patients are capable of inhibiting growth of (and destroying) the same individuals' neoplastic cells in vitro. As will be discussed in another presentation at this symposium by Dr Ingegerd Hellström, we have evidence that the progressive tumor growth in vivo is facilitated by the occurrence in the serum of tumor-bearers of blocking factors, presumably antibodies of the 7s typs, which can in a specific way protect the neoplastic cells from destruction by the immune lymphocytes.

It appeared likely to us that the organism's ability to form blocking serum factors (antibodies?) may play a useful role under some conditions not involving cancer, and that this may explain why such an ability has been preserved by evolution. Previous studies by Kaliss, Batchelor, Fitch and others on the in vivo phenomenon of immunological enhancement also indicated that this may be the case. For that reason, we have performed colony inhibition studies in several different systems to search for the co-existence of cell-mediated immunity and blocking serum factors.

The first system studied concerns the role of blocking serum factors in pregnancy. BALB/c mice which were pregnant with C3H males (once or repeatedly) were used as lymph node cell donors in the experimental group, BALB/c females pregnant with BALB/c being used in the controls. Normal embryonic C3H cells from approximately 18 days old embryos were used as targets. It was found that BALB/c mice pregnant with C3H contained lymphocytes capable of destroying cultivated C3H target cells, and that serum from the same pregnant BALB/c mice could protect the target cells against this destructive effect; no such immune lymphocytes or protective serum factors were found when studying control BALB/c females pregnant to BALB/c males.

The second system studed involved radiation induced canine chimeras. These studies which were carried out in collaboration with Drs. R. Storb and E.D. Thomas, were conducted with dogs which had received approximately 1200 r of whole body x-irradiation and been repopulated with foreign bone marrow. Those dogs studied by us had survived the initial period of greatest risk to die from graft versus host disease and were in good health; they had been maintained varying periods of time following repopulation, the longest survivor 8 years. Cytogenetic as well as serological studies had confirmed that the dogs were, indeed, repopulated with the foreign marrow. It was found that peripheral blood lymphocytes from a repopulated dog (which were of donor origin) inhibited colony formation of the same dog's cultivated skin fibroblasts (which were of recipient origin) and that serum from the same repopulated dogs could specifically protect against that effect. Our data thus indicated that the protective serum molecules (antibodies?) may have played a role as a gruard against graft versus host reaction in vivo.

Our third approach concerned the classical phenomenon of allograft tolerance. This work was conducted in collaboration with Dr. A.C. Allison. CBA as well as A mice were injected as newborn with A x CBA tissue and later found to have become tolerant to the foreign antigens (so that A mice were tolerant to CBA and the CBA mice tolerant to A) according to skin grafting experiments. We could demonstrate that lymph node cells from A mice tolerant to CBA were cytotoxic to cultivated CBA target cells (but not to A cells) and that serum from the same mice (but not from controls) protected against that effect. The analogous type of data was obtained by studying lymph node cells from CBA mice tolerant to A. Our data thus indicated that blocking serum factors may play an important role in the establishment of allograft tolerance; they do, obviously, not exclude that a "true" tolerance with actual absence of lymphoid clones able to react may exist under certain other conditions than those studied.

Finally, we have investigated human patients to whom allogeneic kidneys have been transplanted, to search for cell-mediated immunity against alloantigens of donor origin and for blocking serum factors having the same specificity. These studies have shown that patients who have received a foreign kidney graft and do clinically well, as well as those patients who have rejection crisis, both have a cell-mediated immunity to donor derived graft antigens, as detected by the colony inhibition assay. The former groups of patients, but not the latter one, have blocking serum factors protecting against that immunity.

The findings obtained may explain why allogeneic inhibition is not seen in chimeric animals and counteract the criticism earlier raised against the allogeneic inhibition concept, based on the assumption that this phenomenon is not seen in chimeras or in allophenic mice. More importantly still, these findings may have some relevance with relation to the clinical problem of organ grafting and with regard to our understanding about immunological mechanisms involved in graft rejection. This will be discussed.

MC誘発自家癌の拒絶における細胞性免疫

菊地浩吉（北大・医・病理）
チェスター M. サウザム（スローン・ケッタリング癌研）

Tumor-Specific Cell-Mediated Immune Resistance to Autochthonous Rat Tumors in the Primary Host.

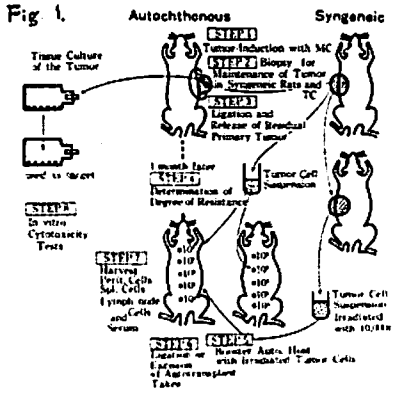
KOKICHI KIKUCHI (Department of Pathology, Hokkaido University School of Medicine) and CHESTER M. SOUTHAM (Sloan-Kettering Institute for Cancer Research)

Tumor-specific resistance to methylcholanthrene-induced tumors in inbred Lewis rats was demonstrated by transplanting autochthonous tumor cells in the primary host and by admixing tumor cells with lymphoid cells from the primary host.

The degree of in vivo resistance in the primary host was expressed by the ratio of the minimum number of tumor cells (MTD) required for the positive take in the autochthonous host as compared with untreated syngeneic rats. The procedure is schematically illustrated in Fig. 1.

For in vitro demonstration, three techniques were used; cytolytic plaque production, release of ^{51}Cr label and cinemicrography.

Fig. 1.



Fifty-two tumor lines were transplantable to syngeneneic rats and in vivo transplantation resistance of 42 primary hosts to each autologous tumors was investigated. Histopathologic appearence of host responses was also studied. The tumors were designated HAT (highly antigenic tumor), MAT (moderately antigenic tumor) and LAT (low antigenic tumor) according to the antigenicity as judged by ratio of MTD in autologous host to MTD in untreated syngeneic rats. It was confirmed that 6 lines of tumors were HAT (the ratio was 10^5-10^4:1), 13 were MAT (10^3-10^1:1) and 23 were LAT (10^0:1).

Out of 26 tumor cell lines which were cultured satisfactorily enough for experiments, 15 autochthonous hosts could be used as sources of immunocytes and serum for in vitro cytotoxicity tests.

Table 1. Fraction of tests causing destruction of tumor cells by autochthonous and untreated syngeneic lymphoid cells with the plaque formation-teet

Target \ Lymphoid cells		Autochthonous			Untreated Syngeneic		
		PC	SnC	LNC	PC	SpC	LNC
HAT	L-1	8/8	5/8	0/2	3/19	2/11	-
	L-28	4/4	-	-	0/4	-	-
MAT	L-33	0/6	0/4	0/4	0/11	0/3	-
	L-26	2/8	6/6	-	2/11	3/10	-
LAT	L-4	6/6	6/6	-	0/6	-	-
	L-13	0/8	0/4	-	0/8	-	-
	L-36	0/1	1/2	-	2/2	2/2	-

1) complement was not added
2) PC,neritoneal cells; SnC,spleen cells; LNC,lymnh node cells

Both cytolytic plaque technique and cinemicrography revealed that tumor cells were frequently destroyed by lymphoid cells from autochthonous hosts. Incubation of the cells for 24-48 hours was required for the reaction. Immunocytes from non-immune syngeneic rats only rarely destroyed tumor cells. The reaction were not influenced by addition of phytohemagglutinin or complement.

In the cinemicrographic studies with highly antigenic tumors the population of target tumor cells decreased gradually by admixing autochthonous peritoneal cells. The destruction of target cells began after about 12 hours, became more striking after 24 hours and completed after 48 hours. In contrast, constant propagation of target cells was observed in the tumor cells admixed with non-immune syngeneic peritoneal cells. We often observed intimate contact of peritoneal cells with a target cell was followed by death of the target cell, however, it was hard to be certain whether the aggregation of the lymphoid cells necessarily preceded the damage of the target cell. Death of the target tumor cells, preceded by retraction of cytoplasmic processes and decreased activity of intracellular movement, was followed by detatchment of cells from the glass.

Quantitative studies of cytotoxic effects of lymphoid cells and serum were performed with ^{51}Cr release cytotoxicity test. The cytolytic effect of lymphoid cells or serum was expressed by "net release [= (release in reaction tube spont.release)/(maximum release - spont.release)]". Peritoneal cells from the primary host, that had acquired resistance to their tumor were most effective in destroying tumor cells (Table 2). Autologus spleen cells were less effective and lymph node cells had little or no effect. Autologous sera had almost no effect in the presence of complement.

Limitted data suggested that the cytolytic effect was specific for individual tumors.

Relationship between *in vivo* transplantation resistance and *in vitro* tests was studied. The frequency of plaque formation with autochthonous immunocytes did not always parallel the degree of *in vivo* transplantation resistance (Table 1). In the ^{51}Cr release studies, it can be seen that there was a direct relationship between *in vivo* resistance and *in vitro* cytotoxicity of autochthonous peritoneal cells (Fig.2). These facts imply that the estimation of immunologic state of resistance in cancer patients against the autochthonous cancer may be possible by *in vitro* tests.

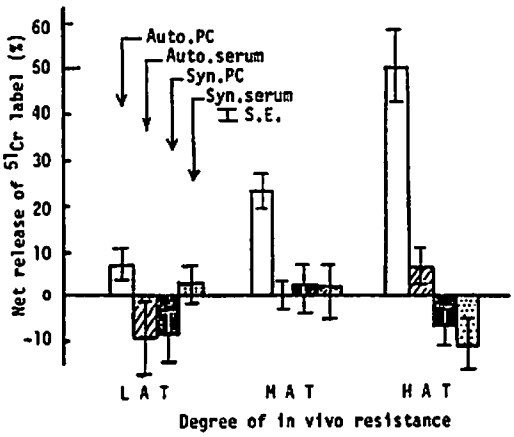
The mechanism by which lymphoid cells destroy the target cells have not been completely elucidated. Time-lapse phase contrast cinemicrography revealed striking destruction of target tumor cells as previously described, however, it was not certain whether or not contact of lymphoid cells on target cells is indispensable for cytolysis. To solve the problem, an assembly was devised which permitted growth of target cells directly on oneside of the Millipore membrane and lymphoid cells on the other side. Although the existence of cytotoxic substances diffusible through HA Millipore membrane was proved in some experiments, negative results were also frequently observed. This may suggest that lymphoid cells produced a diffusible substance which may be tightly bound with lymphoid cells and may be different from typical antibody.

(This work was supported by grants #T404 and T229 from the American Cancer Society and #5 ROI CA-07863 and CA-08748 from the National Institute of Health)

Table 2. Net-release of ^{51}Cr from target-tumor cells after incubation for

	24 hrs	48 hrs
with autologous		
PC	28.9	47.8
SpC	8.2	34.6
LNC	-2.5	17.6
Serum	-13.3	1.5
with syngeneic		
PC	-10.4	-3.6
SpC	-6.6	1.6
LNC	-9.0	-28.7
Serum	-6.5	-5.3

Fig 2. Relationship between *in vitro* cytotoxicity of lymphoid cells and degree of *in vivo* resistance to the autochthonous tumor



リンパ球と腺癌細胞との培養内相互作用

石橋幸雄*, 芦川和高*, 勝田　甫†, 高岡聰子†
(* 東大医科研・外科,　† 東大医科研・癌細胞)

Interaction in culture between tumor and lymphoid cells of the isologous mouse.

YUKIO ISHIBASHI*, KAZUTAKA ASHIKAWA*, HAJIM KATSUTA†, & TOSHIKO TAKAOKA† (* Department of Surgery, and † Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo)

The interaction between transplantable ascites mammary carcinoma MM2 cells of C3H/He mice and thymocytes or lymphocytes of mesenterial lymph nodes of untreated or tumor-bearing isologous mice of the same sex was investigated by means of tissue culture.

When cultured alone in the medium consisting of 20% inactivated calf serum and 80% synthetic medium 199, all of these cells showed little change in cell number during 7 days of incubation at 37°C.

In the mixed culture of MM2 cells and thymocytes of untreated mice the number of MM2 cells was kept unchanged, whereas that of thymocytes decreased in every case of experiment (Figs. 1 and 2). This change did not depend upon the mixture rate of both cells. Similar result was also obtained in the mixed culture of MM2 cells and thymocytes derived from tumor-bearing mice. In the mixed culture with histiocytes, thymocytes of untreated mice did not show any decrease in number.

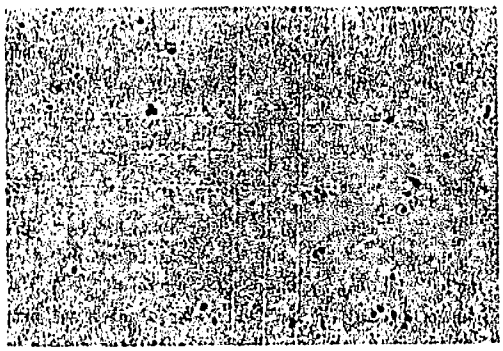


Fig. 1. Thymocytes of untreated C3H/He mice in a haemocytometer after 7 days of cultivation.

To examine why the number of thymocytes was decreased in the mixed culture with MM2 cells, changes in the cells of the mixed culture were investigated by the use of cinemicrography.

In the control culture of thymocytes and lymphocytes of mesenterial lymph nodes from untreated C3H/He mice or MM2 cells alone, all of these cells showed active movement. In the mixed culture with MM2 cells, however, it was evidently demonstrated that both of thymocytes and lymphocytes of mesenterial lymph nodes from untreated C3H/He mice were all phogocytosed by MM2 cells (Fig. 3).

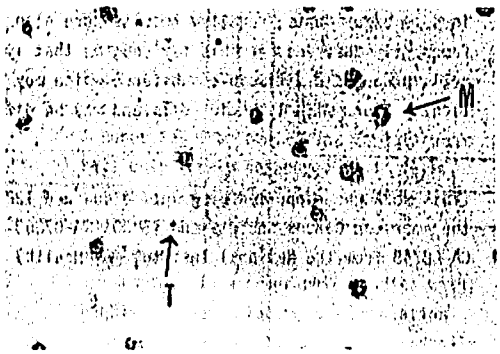


Fig. 2. Thymocytes (T) of untreated mice and MM2 cells (M) in a haemocytometer after 7 days of mixed culture. Marked decrease in number of thymocytes is noticed.

Thymocytes from the MM2-bearing mice were mixed with MM2 cells in culture 3 weeks after the subcutaneous transplantation and were observed by cinemicrography for the period of almost 4 weeks. Most of the thymocytes were found

to have been phagocytosed by MM2 cells within 1 week, only MM2 cells being remained in the culture after 3 weeks.

In the mixed culture of MM2 cells and lymphocytes obtained from mesenterial lymph nodes of C3H/He mice sensitized beforehand with DOC-extracted antigen of MM2 cells, all of the lymphocytes were phagocytosed by the tumor cells within 1 week. The observation in 2 or 3 weeks revealed the existence of MM2 cells alone in the culture. These tumor cells showed very active behavior and, on intraperitoneal back-transplantation into mice, produced tumors in the mice all of which died of tumor by 38 days. The phagocytosis of erythrocytes by MM2 cells was hardly detected, while some of the cells obtained from spleen tissue were phagocytosed by the tumor cells.

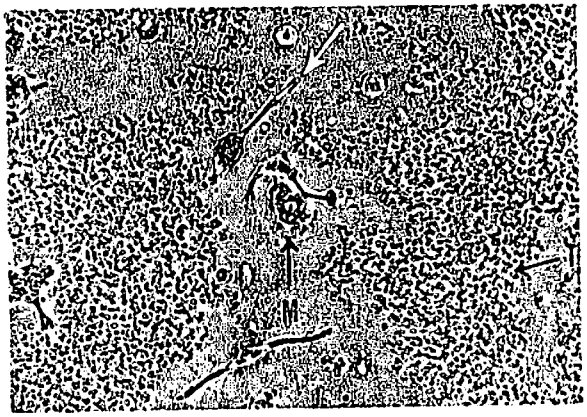


Fig. 3. The mixed culture of thymocytes (T) from untreated mice and MM2 cells (M) in 13 days. A white arrow indicates the phagocytosis of a thymocyte by a MM2 tumor cell.

It should be emphasized that no tumor cells were observed to have undergone degeneration or necrosis due to the phagocytosis of these lymphoid cells including those derived from sensitized mice.

Lymphoid cells of sensitized animals have been convinced to be cytocidal against tumor cells, and this hypothesis has been regarded as an indirect evidence of cellular immunity.

In this paper, however, lymphoid cells did not damage tumor cells but, contrarily, were phagocytosed by them. The reason is obscure, but it tells us the importance of the observation of dynamic morphology of cells under these environments.

CONTENTS (continued)

免疫生物学シンポジウム4．編集・発行：免疫生物学研究会（事務局．京都市左京区北白川追分町 京都大学理学部動物学教室内 村松 繁気付． 電話 075—771—8111 内線 3296. 振替口座 京都 15350 三菱銀行出町支店気付）

SYMPOSIUM ON IMMUNOBIOLOGY 4

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1970. 11. 29~30 — Nagoya

JAPANESE SOCIETY FOR IMMUNOBIOLOGY

第4回
免疫化学シンポジウム
免疫生物学シンポジウム
プログラム

THE 4TH
SYMPOSIUM
ON
IMMUNOCHEMISTRY
AND
IMMUNOBIOLOGY

昭和45年11月28、29、30日
於　愛知県勤労会館・名古屋
NOV. 28, 29, 30, 1970

NAGOYA

免疫化学シンポジウム

第1日　11月28日（土）午前

9.25-9.30 開会の辞　伊藤洋平（愛知県がんセンター）

Opening remarks Yohei Ito (Aichi Cancer Center)

1. 9.30-10.00 ウサギIgGのA2アロタイプ

柿沼光明（北大結核研究所）

Studies on the specificity of allotype A2 of rabbit immunoglobulin G.

Mitsuaki Kakinuma (Research Institute for Tuberculosis, Hokkaido University, Sapporo)

2. 10.00-10.30 免疫グロブリンの Idiotypic Determinants

松岡雄治・高田 優・北川正保（阪大・医・癌研）

Idiotypic determinants of immunoglobulins.

Yuji Matsuoka, Masaru Takata and Masayasu Kitagawa (Institute for Cancer Research, Osaka University Medical School, Osaka)

3. 10.30-11.00 IgAミエローマ蛋白の一次構造

野津祐三・岡田吉美（植物ウイルス研）、千谷晃一（阪大・蛋白研）、渡辺信一郎・原 弘道・北川正保（阪大・医・がん研）

On the chemical structure of IgA myeloma protein of lamda type.

Yuzo Nozu, Yoshimi Okada, Koichi Titani*, Shinichiro Watanabe**, Hiromichi Hara** and Masayasu Kitagawa**

Institute for Plant Virus Research, Chiba, *Institute for Protein Research, Osaka University, **Institute for Cancer Research, Osaka University, Osaka)

4. 11.00-11.30 Bence Jones 蛋白の側鎖の状態

東逸親・浜口浩三（阪大・現・生物）右田俊介（金沢大・がん研）

States of side chains in Bence Jones proteins.

Takachika Azuma*, Kozo Hamaguchi* and Shunsuke Migita**

(*Department of Biology, Faculty of Science, Osaka University, Osaka, **Cancer Research Institute, Kanazawa University, Kanazawa)

5. 11.30-12.00 分泌型IgAの免疫化学的研究（II）遊離およびIgA結合性Secretory Pieceの分離、精製とその性質について

小林邦彦・平井秀松（北大・医・第一生化学）

Studies on human secretory IgA (II): Comparative studies of IgA-bound secretory piece and free secretory piece.

Kunihiko Kobayashi, and Hidematsu Hirai (Department of Biochemistry, Hokkaido University School of Medicine, Sapporo)

6. 12.00-12.30 イヌおよびラットのhomocytotropic antibody－その物理化学的、免疫化学的性質－

藤本重義・奥村 康・多田富雄（千葉大学・第二病理）

Homocytotropic antibodies in the dog and rat — Physicochemical and immunochemical characterizations —.

Shigeyoshi Fujimoto, Ko Okumura and Tomio Tada (Department of Pathology, School of Medicine, Chiba University, Chiba)

12.30-13.30 昼 休 み （世話人会、特別会議室）

第１日　　１１月２８日（土）　午　後

13.30-13.50　免疫化学シンポジウム総会

7. 13.50-14.20　ウサギIgM抗体結合部位の抗原との親和性

尾上 薫・岸本忠三（九大・歯・生化学）

The antigen binding properties of rabbit IgM antibody.

Kaoru Onoue and Tadamitsu Kishimoto (Department of Biochemistry, Kyushu University School of Dentistry, Fukuoka)

8. 14.20-14.50　ステム・ブロメライン酵素活性の抗体による中和

飯田重毅・佐々木実・村地孝（名市大・医・生化学）

The mechanism of inhibition of stem bromelain by its specific antibodies.

Shigeki Iida, Makoto Sasaki and Takashi Murachi (Department of Biochemistry, School of Medicine, Nagoya City University, Nagoya)

9. 14.50-15.20　モルモット抗卵アルブミン抗体の沈降反応の異常性について

中村享・大船一信・田元浩一・上原和子・小山次郎（北大・薬、徳島大・医）

Immunochemical studies on guinea pig nonprecipitating antibody to ovalbumin.

Tohoru Nakamura, Kazunobu Ohune, Koichi Tamoto, Kazuko Uehara* and Jiro Koyama (Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, *Medical School, Tokushima University, Tokushima)

10. 15.20-15.50　IgMサブユニットの溶血活性と溶血反応におけるinter-chainジスルフイド結合の必要性について

池田正春・岸本忠三・尾上 薫（九大・歯・生化学）

Properties and hemolytic activity of the reduction products of rabbit IgM antibody.

Masaharu Ikeda, Tadamitsu Kishimoto and Kaoru Onoue (Department of Biochmistry, Kyushu University School of Dentistry, Fukuoka)

11. 15.50-16.20 ヘビ血清中の異種赤血球溶血成分

川口 進・村松 繁（京大・理・動物）三橋 進（群大・医・微生物）

Studies on the natural hemolytic activity of snake serum.

S.Kawaguchi*, S.Muramatsu* and S.Mitsuhashi**

(*Department of Zoology, Faculty of Science, Kyoto University, Kyoto, **Department of Microbiology, School of Medicine, Gunma University, Maebashi)

12. 16.20-16.50 蝸牛 Helix pomatia 中に存在する抗A凝集素の物理化学的性状

石山昱夫・高津光洋（東大・医・法医）

Purification of helix anti-A agglutinin and its specificities as serologically acitive aeagent.

Ikuo Ishiyama and Akihiro Takatsu (Department of Legal Medicine, Faculty of Medicine, University of Tokyo, Tokyo)

13. 16.50-17.20 肝リボソームと抗カタラーゼとの結合

東 真彦・工藤尚子（昭和大・薬・生理化学）

Binding of anticatalase by rat liver ribosomes.

Tokuhiko Higashi and Hisako Kudo (Department of Physiological Chemistry, School of Pharmaceutical Sciences, Showa University, Tokyo)

14. 17.00-17.50 リゾチームの immunodominant group とその抗体

藤尾 啓・坂戸信夫・天野恒久（阪大・微研・免疫化学）

Two distinct immunodominant groups of lysozyme and their respective antibodies.

Hajime Fujio, Nobuo Sakato and Tsunehisa Amano (Department of Immunochemistry, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka)

第２日　　１１月２９日（日）　午前

15. 9.10-9.40 α1→2結合を含むDextran NRRL B1397の免疫化学的研究

鳥居光雄・榊原京子（阪大・微研・免疫化学）

Immunochemical studies on Dextran NRRL B1397 containing α 1-2 linkages.

Mitsuo Torii and Kyoko Sakakibara (Department of Immunology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka)

16. 9.40-10.10 哺乳動物細胞膜糖脂質の抗原性（特にホルスマン・ハプテンについて）

内貴正治（信大・医・順応研・生化学）上村英夫（信大・医・衛検）

Immunochemical properties of glycolipids in mammalian cell membrane (Studies on Forssman globoside).

M. Naiki and H. Kamimura (Department of Biochemistry, Institute of Adaptation Medicine, Shinshu University, Matsumoto)

17. 10.10-10.40 C3Hマウス・ウサギγGの系における免疫寛容の「終止」の機構について

藤原道夫（東大・医科研・免疫）

On the mechanism of the termination of immunological tolerance.

Michio Fujiwara (Institute of Medical Science, University of Tokyo, Tokyo)

18. 10.40-11.10 抗DNP系における免疫寛容の誘発と担体特異性効果の欠除

藤本輝夫・内海爽・天野恒久（阪大・医・細菌学）

Induction of immunological paralysis to DNP group and the absence of carrier specific effect in the paralysis induction.

T. Fujimoto, S. Utsumi and T. Amano (Department of Bacteriology, Osaka University Medical School, Suita)

19. 11.10-11.40 Hapten－Carrier系の抗原認識に及ぼす抗hapten, 抗Carrier抗体の調節作用－抗Carrier抗体の抗hapten抗体産生に及ぼす抑制効果－

高津聖志・浜岡利之・北川正保（阪大・医・がん研）

Regulatory effect of anti-hapten and anti-carrier anti-bodies on the recongnition of antigenic determinants in hapten-carrier system － Suppressive effect of anti-carrier antibody on the anti-hapten antibody response.

K. Takatsu, T. Hamaoka and M. Kitagawa (Institute for Cancer Research, Osaka University Medical School, Osaka)

20. 11.40-12.10 ラット homocytotropic antibody （HTA）産生の特異的抑制機序

奥村 康・谷口 克・多田富雄（千葉大・医・第二病理）

Regulatory mechanisms of homocytotropic antibody formation in the rat.

Ko Okumura, Masaru Taniguchi and Tomio Tada (Department of Pathology, School of Medicine, Chiba University, Chiba)

閉会の辞 右田俊介（金沢大学がん研）
佐々木実（名市大医生化）

Closing remarks Shunsuke Migita
(Cancer Research Institute, Kanazawa Universiy)

Makoto Sasaki
(School of Medicine, Nagoya City University)

免疫生物学シンポジウム

第2日　11月29日（日）午后

開会の辞　伊藤洋平（愛知県がんセンター）

Opening remarks　Yohei Ito (Aichi Cancer Center)

Session I　2.00-6.00 p.m.

1. Introductory remarks: 胸腺依存リンパ球の動態
   花岡正男（京大・ウイルス研・病理）
   Thymus-dependent lymphocytes in lymph node and spleen.
   Masao Hanaoka (Department of Pathology, Institute for Virus Research, Kyoto University, Kyoto)
2. 鳥類の胸腺とファブリシウス嚢の機能
   佐藤孝二（名大・農・畜産）
   Immunological functions of the thymus and bursa of Fabricius.
   Koji Sato (Department of Animal Physiology, Nagoya University, Nagoya)
3. 無菌動物のリンパ系
   宇野裕（名大・医・無菌動物研究施設）
   The lymphatic system of germfree animals.
   Yutaka Uno (Germfree Life Research Laboratory, School of Medicine, Nagoya University, Nagoya)
4. Homeostasis of cell-mediated immunity.
   David S. Nelson (Department of Bacteriology, the University of Sydney, Sydney)
5. 感作リンパ球の抗体産生における役割
   野本亀久雄（九大・医・細菌）
   The role of sensitized lymphocytes in antibody production against the same antigens.
   Kikuo Nomoto (Department of Bacteriology, School of Medicine, Kyushu University, Fukuoka)

6. 白血球抗原の免疫系統発生学的研究

辻　公美（慶大・医・放医）

Phylogenic study of leucocyte antigen (HL-A antigen).

Kimiyoshi Tsuji (Department of Radiology, Keio University School of Medicine, Tokyo)

7. Surface immunoglobulin-moieties on lymphoid cells.

Eva Klein (Institute for Tumor Biology, Karolinska Institute, Stockholm)

8. Friend Virus 感染ラットのラント症候群

武市紀年・葛巻 進・小林 博（北大・医・癌研・病理）

Runting syndrome of the rat infected with Friend virus.

Noritoshi Takeichi, Noboru Kuzumaki and Hiroshi Kobayashi (Cancer Institute, Hokkaido University School of Medicine, Sapporo)

9. ウイルス感染における細胞性免疫（アデノウイルス感染系における検討）

浜田忠弥・中島節子・植竹久雄・（京大・ウイルス研）

Cell-mediated Immunity in Adenovirus infection.

Chuya Hamada, Setsuko Nakajima and Hisao Uetake (Institute for Virus Research, Kyoto University, Kyoto)

10. マウスの3型レオウイルス感染症における抗リンパ球血清の影響

井田士朗・日沼頼夫（東北大・歯・微生物）

Effect of antilymphocyte serum on reovirus infection of mice.

Shiro Ida and Yorio Hinuma (Department of Microbiology, Tohoku University School of Dentistry, Sendai)

11. 骨髄細胞の抗原反応性へのＡＬＳの影響について

新井義夫・辻 正・野呂瀬嘉彦・市川洋一（日医大・微生物－免疫）

Influence of ALS on the antigen-reactivity of bone-marrow cell (guinea pig).

Yoshio Arai, Tadashi Tsuji, Yoshihiko Norose and Yoichi Ichikawa (Department of Microbiology and Immunology, Nippon University Medical School, Tokyo)

12. Lymphoid cell surface antigens and cell-mediated immunity.

D. Bernard Amos (Department of Microbiology amd Immunology, Duke University, Durhan)

ま と め

懇 親 会 (Reception) 7.00-9.00 p.m.

第３日　　１１月３０日（月）午前

Session II　9.10-11.00 a.m.

13. Introductory remarks: Allogenic inhibition and cell-mediated immunity.

Karl E. Hellström (Department of Pathologt, School of Medicine, University of Washington, Seattle)

14. 同種腫瘍細胞に対する試験管内細胞性免疫反応

橋本嘉幸（東京生化学研）

In virto cell-mediated immune reaction against allogenic tumor cells.

Yoshiyuki Hashimoto (Tokyo Biochemical Research Institute, Tokyo)

15. ラットにおけるlocal GVH反応

池田久美・小室勝利・相沢 幹（北大・医・病理）

Local graft-versus-host reaction in rats.

Hisami Ikeda, Katsutoshi Komuro and Miki Aizawa (Department of Pathology, Hokkaido University School of Medicine, Sapporo)

16. ニワトリの血液型とＧＶＨ反応

藤尾芳久（名大・農・家畜育種）

Relationship between blood groop gene B and graft-versus-host reaction activity in the chicken.

Yoshihisa Fujio (Laboratory of Animal Genetics, Faculty of Agriculture, Nagoya University, Nagoya)

17. 腫瘍同種移植免疫における体液性抗体の役割

湯徳正道・妹尾八郎・北川正保（阪大・医・癌研）

Role of humoral antibodies in tumor allograft immunity.

Masamichi Yutoku, Hachiro Senoh and Masayasu Kitagawa (Institute for Cancer Research, Osaka University, Osaka)

(11)

18. 皮膚反応惹起因子（ＳＲＦ）並びにマクロファージ遊走阻止因子（ＭＩＦ）の種属特異性

吉田　彪・綿貫まつ子・橋本達一郎（予研・結核部）

Species specificity of skin reactive factor (SRF) and macrophage migration inhibitory factor (MIF).

Takeshi Yoshida, Matsuko Watanuki and Tatsuichiro Hashimoto (Department of Tuberculosis, The National Institute of Health, Tokyo)

ま と め

Session III 11.00-12.00 a.m.

19. 腫瘍特異抗原

北川正保（阪大・医・癌研）

Tumor specific antigens.

Masayasu Kitagawa (Institute for Cancer Research, Osaka University, Osaka)

20. 感作腹腔内細胞による腫瘍移植抗原の識別とその変化

石館 基（癌研）

Antigenic specificity of hepatoma cell lined recongnized sensitized peritoneal lymphoid cells.

Motoi Ishidate, Jr. (Cancer Institute, Tokyo)

21. マウス乳癌の腫瘍特異抗原の精製と化学分析

入江礼子・三井宏美・片岡建之・西岡久寿弥（国立ガンセンター研究所・ウイルス部・生物部・東京大学薬学部）

Chemical analysis of further purified tumor specific antigens of mouse mammary tumors.

Reiko Furuse Irie, Hiromi Mitsui, Takeshi Kataoka and Kusuya Nishioka (Virology Division and Biology Division, National Cancer Center Research Institute, and Faculty of Pharmaceutical Science, University of Tokyo, Tokyo)

22. Friend virus によるラット細胞の異物化

小林 博（北大・医・ 癌研）

Xenogenization of rat cells by infection with friend virus.

Hiroshi Kobayashi (Cancer Institute, Hokkaido University School of Medicine, Sapporo)

昼 休 み （世話人会、特別会議室）

第３日　　１１月３０日（月）午後

免疫生物学研究会総会　　1:00－2:00 p.m.

Session IV　2.00-5.00 p.m.

23. Cell-mediated immunity in cancer patients.
C. M. Southam (Sloan-Kettering Institute for Cancer Research, New York)

24. Cell-mediated immunity against tumors.
Ingegerd Hellström (Department of Microbiology, University of Washington, Seattle)

25. Cell-mediated immunity と allogenic inhibition について
折田薫三・小長英二・大西信行・寺田紀彦・国　欣明・田中早苗
（岡山大・医・外科）
Cell-madiated immunity and allogenic inhibition of tumor bearing hosts.
Kunzo Orita, Eiji Konaga, Nobuyuki Ohnishi, Norihiko Terada, Yoshiaki Kokumai and Sanae Tanaka (Department of Surgery, Okayama University, Okayama)

26. Studies on the role of blocking serum factors (antibodies) under some conditions not involving neoplasia.
Karl Erik Hellström (Department of Pathology, University of Washington, Seattle)

27. ＭＣ誘発自家癌の拒絶における細胞性免疫
菊地浩吉（北大・医・病理）、チェスター・Ｍ・サウザム（スローン・ケッタリング癌研）
Tumor-specific cell-mediated immune resistance to autochthonous rat tumors in the primary host.
Kokichi Kikuchi (Department of Pathology, Hokkaido University School of Medicine, Sapporo) and Chester M. Southam (Sloan-Kettering Institute for Cancer Research, New York)

28. リンパ球と腫瘍細胞との培養内相互作用

石橋幸雄・芦川和高（東大・医科研・外科）、勝田甫・高岡聡子（東大・医科研・癌細胞）

Interaction in culture between tumor and lymphoid cells of isologous mouse.

Yukio Ishibashi*, Kazutaka Ashikawa*, Hajime Katsuta** and Toshiko Takaoka** (Department of Surgery*, Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Tokyo)

閉会の辞　吉田孝人・森田敏照（愛知県がんセンター）

Closing remarks　Takato Yoshida, Toshiteru Morita (Aichi Cancer Center)

お詫び： Dr. George Klein はスエーデンに２７日迄に帰国せねばならなくなりましたので予定をプログラムに示したように変更しました。

連　絡：
1. 参加者は参加費５００円を受付の係へお払い下さい。
2. スライドは３５㎜版のみとします。３０分前迄にスライド受付へお渡し下さい。
プロジエクターは原則として１台，但し必要であれば２台使用することも出来ます。
3. ２９日，会の終了後，懇親会を行います。
参加御希望の方は会費８００円を受付でお払い下さい。

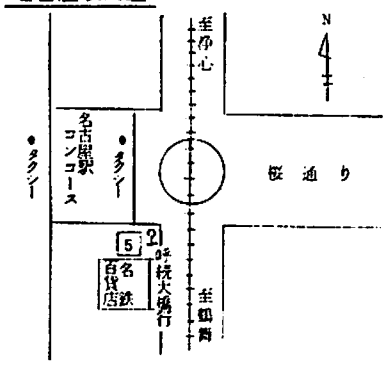
会期前の
問合せ　世話人代表　伊藤洋平
名古屋市千種区田代町鹿子殿８１－１
愛知県がんセンター．ウイルス部
電話　052-762-6111 内線731，730

会期中の
問合せ　事務局：愛知県勤労会館　特別会議室

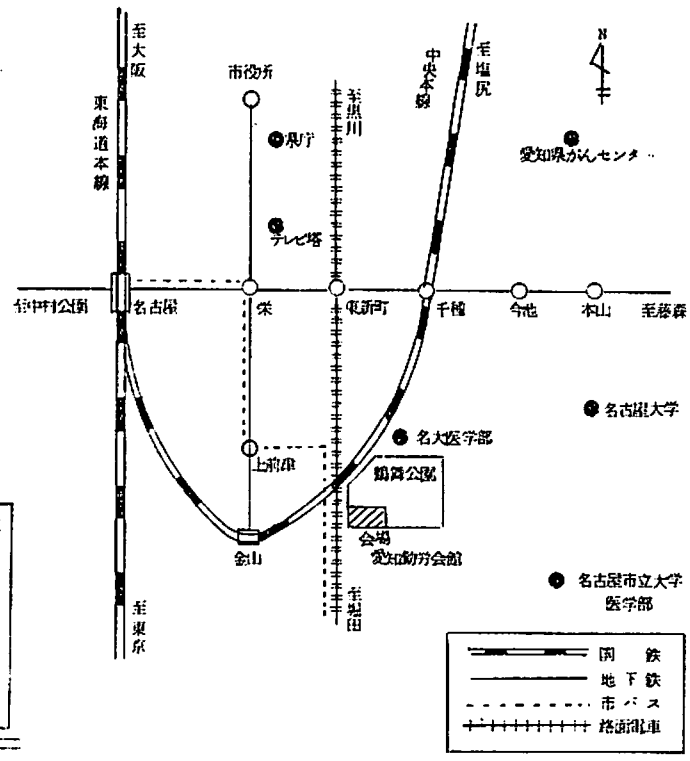
お知らせ　免疫実験操作法テキスト（150ページ，700円）免疫化学シンポジウム，免疫生物研究会
補体シンポジウム共同発行を当日会場にて御希望の方に配布いたします。

問合せ先：名古屋市瑞穂区瑞穂町　名古屋市立大学医学部
生化学教室　佐々木　実

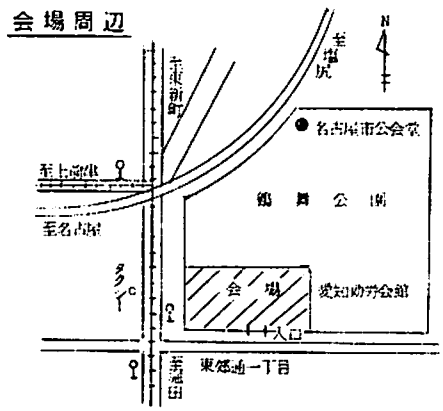
名古屋駅周辺



会場案内



会場周辺



会　場　愛知県勤労会館
名古屋市昭和区鶴舞町４５の１　電話 名古屋 733-1141

交通機関　バス：名古屋駅東口バス５番のりばより４１系呼続大橋行にて約２５分，東郊通一丁目下車，東へ５０米．（３０円）
タクシー：名古屋駅前より約１５分，（３００円）