

A NEW DIFFERENTIATION ANTIGEN (FT-1) SHARED WITH FETAL THYMOCYTES AND LEUKEMIC CELLS IN THE MOUSE*

BY MASATAKA KASAI, TOHRU TAKASHI, TOSHITADA TAKAHASHI,**
AND TOHRU TOKUNAGA

*From the Department of Tuberculosis, National Institute of Health, Shinagawa-ku, Tokyo 141, and **Laboratory of Cell Biology, Aichi Cancer Center Research Institute, Nagoya 464, Japan*

The recent development of hybridoma technology has enlarged our understanding of cell surface molecules, especially those of lymphoid organs. T cell leukemia, which is a common neoplasm of the mouse, and its normal counterpart, the thymocyte, have been a major focus for studying cell surface antigens (1). It has been generally accepted that, in the mouse embryo, thymic stem cells are derived from either the yolk sac or the fetal liver. Shortly after the stem cells enter into the thymus, they begin to proliferate and give rise to thymocytes in the cortex and medulla, where they can be divided into functional subclasses of T lymphocytes having a wide repertory of antigen specificity (2). Serological analysis has revealed that this process of differentiation of thymic lymphocytes involves a number of marked changes in surface antigens, Thy-1, Lyl-1,2,3, TL and other T cell differentiation antigens (3-5). Almost all of these antigens, except for the TL systems, are quantitative rather than qualitative features. The presence of TL antigen is restricted to adult thymocytes and leukemias of thymic origin, and the mechanism of regulation of gene coding for this antigen is of particular interest. However, no report has yet appeared that describes the antigens expressed predominantly on fetal thymocytes and leukemia cells.

We previously reported that the receptor for *Dolichos biflorus* agglutinin (DBA),¹ which is specific for terminal nonreducing α -linked *N*-acetyl D-galactosamine (D-GalNAc) residues (6), is selectively expressed on mouse fetal thymocytes and some leukemia cells (7, 8). The immunofluorescence study indicated that a majority of the cells from the mouse embryo thymus (13 d gestation) were brightly stained with fluorescein isothiocyanate (FITC)-conjugated DBA lectin, and the proportion of such cells sharply decreased with the increase in gestation time. Unlike other already defined T cell antigens, the DBA receptor is not

This work was supported in part by a grant for scientific research from the Ministry of Education, Science and Culture of Japan, and by a Grant-in-Aid from the Ministry of Health and Welfare of Japan.

* Supported in part by a grant for scientific research from the Ministry of Education, Science and Culture of Japan, and by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan.

¹ Abbreviations used in this paper: DBA, *Dolichos biflorus* agglutinin; D-GalNAc, D-galactosamine; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; FT-1, fetal thymus antigen-1; HAT, hypoxanthine, aminopterin, and thymidine; MEM, minimal essential medium; NK, natural killer; PEG, polyethylene glycol; SDS, sodium dodecyl sulfate.

expressed on cells of adult lymphoid tissues including thymus, spleen, lymph nodes, and bone marrow. In this report, production of a mouse monoclonal antibody detecting this receptor on fetal thymocytes is described. Biochemical properties of the antigen recognized, which was provisionally designated fetal thymocyte antigen-1, FT-1, were also investigated.

Materials and Methods

Animals and Leukemias. A/J, AKR/J, BALB/c, CBA/J, C57BL/6, C3H/HeN, DBA/2, NZB, and SJL mice were supplied by the Omura Institute for Laboratory Animals (Zama, Kanagawa, Japan). GR mice were kindly provided by Dr. T. Sakakura, Laboratory of Experimental Pathology, Aichi Cancer Center Research Institute, Nagoya, Japan.

The leukemias used were kindly given to us by Dr. L. J. Old of Memorial Sloan-Kettering Cancer Institute, New York, and Dr. J. Hilgers, The Netherlands Cancer Institute, The Netherlands. YAC-1 and GRSL cultured cell lines were maintained in RPMI 1640 medium supplemented with 5% fetal calf serum (FCS). All other leukemias were carried in the ascites form.

Cell Fusion and Cloning. BALB/c mice were hyperimmunized with 1×10^7 GRSL cells. 3 d after the booster injection, 5×10^7 spleen cells from the mice were fused with X63-Ag8.653 cells in the presence of polyethylene glycol (PEG; mol wt 1,500) (9, 10). Then the cells were plated in a 96-well culture plate (Costar #3596; Costar, Data Packaging, Cambridge, MA) and grown in medium containing hypoxanthine, aminopterin, and thymidine (HAT). 3 wk after the fusion, the culture supernatants were screened in the immunofluorescence study. Cells from these wells were cloned by limiting dilution on feeder layers of BALB/c thymocytes. A clone derived from one well (M6) was used for the study described here. As M6 antibody was determined to be of the IgM isotype, the antibody in the supernatant was precipitated by $(\text{NH}_4)_2\text{SO}_4$, and fractionated by Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden), and used for the experiments.

Metabolic Labeling and Immunoprecipitation. Cells of GRSL, a spontaneous leukemia of GR mice, were suspended at 2×10^6 cells/ml in RPMI 1640 with 5% FCS. [^3H] galactose (10.4 Ci/mmol, Amersham, International, Amersham, U.K.) was added at 50 $\mu\text{Ci/ml}$ and cells were incubated at 37°C in 5% CO_2 for 16 h. The biosynthetic labeling was also carried out by adding 50 μCi of [^{35}S] methionine (1,450 Ci/mmol, Amersham) to 2×10^6 cells in Minimum Essential Medium (MEM) (methionine free) with 2% FCS for 16 h. Cells were washed twice and extracted in 1 ml of lysis buffer (0.5% Nonidet P-40 [NP-40], 10 mM Tris HCl pH 7.5, 150 mM NaCl, 1 mM MgCl_2 , 0.1% NaN_3 , 1 mM phenylmethylsulfonyl fluoride), with gentle vortex mixing for 30 min on ice.

For immunoprecipitation, the lysate was incubated with 20 μl of anti-FT-1 for 2 h at 0°C, and then with 50 μl of goat anti-mouse IgM serum for an additional 10–20 h. The resulting immune complex was mixed with 200 μl of 10% fixed *Staphylococcus aureus* Cowan I strain organisms (Bethesda Research Laboratories, Inc., Gaithersburg, MD) for 60 min at 0°C. The precipitate was washed extensively and then bound antigens were released by boiling for 3 min in sample buffer containing 20% glycerol, 4% sodium dodecyl sulfate (SDS), 120 mM Tris HCl, pH 6.8, and 0.005% bromphenol blue with or without 5% 2-mercaptoethanol.

Immunofluorescence and Cytotoxicity Test. For tissue distribution studies, 1.0×10^6 lymphoid cells were incubated for 30 min at 4°C in 50 μl of MEM containing 5 μg of biotin-conjugated anti-FT-1. The cells were then washed three times and stained for 30 min at 4°C with 1 μg of FITC-avidin in 50 μl of MEM. FITC-monoclonal anti-Thy-1.2 antibody used at a 1:40 dilution was kindly provided by Dr. K. Okumura, University of Tokyo, Tokyo, Japan. 1 μg of FITC-DBA in 50 μl of MEM was used for staining. The absorption test and complement-dependent cytotoxicity assay were carried out according to the method described previously (11).

Isolation of DBA Receptors. DBA receptors were isolated from GRSL cells according to the method described previously (8). Briefly, the detergent lysate of GRSL cells was applied to a DBA-Sepharose 4B column equilibrated with the elution buffer (0.5% NP-

TABLE I
Tissue Distribution of FT-1 Antigen

Tissue (A/J)	Fluorescent staining (%)	Absorption with
Fetal mouse (15th gestation day)		
Thymus	58	NT*
Liver	0	NT
Adult mouse (8 wk)		
Thymus	0	— [‡]
Spleen	0	—
Lymph node	0	—
Bone marrow	0	—
Liver	NT	—
Kidney	NT	—
Brain	NT	—
Control GRSL	100	+ [§]

Various tissues were examined for FT-1 antigen by immunofluorescent staining as described in Materials and Methods. The presence of antigen on adult lymphoid tissues and homogenized liver, kidney, and brain tissues was also determined by an absorption test. 2 vol of diluted M6 antibody, one twofold dilution below its endpoint, and 1 vol of packed, washed cells were mixed and incubated for 30 min at 4°C. After removal of the absorbing cells by centrifugation, the residual cytotoxic activity against GRSL cells was tested.

* NT: not tested.

[‡] -: not absorbed.

[§] +: absorbed.

40, 10 mM Tris-HCl buffer, pH 7.5 with 150 mM NaCl and 1 mM CaCl₂). DBA-Sepharose 4B was prepared by coupling DBA (E. Y. Laboratories, San Mateo, CA) to CNBr (cyanogen bromide)-activated Sepharose 4B (Pharmacia). After the column was washed, the receptors were eluted from the affinity column by 0.1 M *N*-acetyl-galactosamine.

Polyacrylamide Gel Electrophoresis (PAGE) and Fluorography. The DBA receptors were fractionated by 7.5% SDS-PAGE under reducing or nonreducing conditions. After the electrophoresis, the gels were stained with Coomassie Brilliant blue, destained with 10% acetic acid and then processed for fluorography. Briefly, the gels were soaked in 10 vol of 1 M sodium salicylate for 30 min (12) and dried on Whatman 3 MM paper and then exposed to X-ray film (Kodak X-Omat AR) at -70°C.

Two-dimensional Gel Electrophoresis. FT-1 antigens labeled with [³H] galactose were electrophoresed in two dimensions according to the method developed by O'Farrell (13). The first dimension separation was by nonequilibrium pH-gradient electrophoresis. The second dimension SDS gel electrophoresis was carried out in 10–16% acrylamide gradient gel.

Results

Tissue Distribution. Cell hybridization was performed between BALB/c myeloma, X63-Ag8.653 cells, and the lymphocytes of BALB/c mice that were immunized with GRSL cells. One of the hybrids, M6, which reacted with fetal thymocytes but not with adult thymocytes, was subcloned twice by limiting dilution.

Cells from different tissues of A/J mice were incubated with the biotinylated M6 antibody and then stained with FITC-avidin. As summarized in Table I, M6

TABLE II
Change of FT-1⁺ and Thy-1⁺ Cells in the Thymus at Different Embryonic Stages of the A/J Mouse

Gestation day	% Immunofluorescence-positive cells	
	FT-1	Thy-1
13	92	0
14	76	22
16	41	60
18	7	91

Fetal thymocytes positive for FT-1 or Thy-1 were determined by immunofluorescent staining as described in Materials and Methods.

antibody brightly stained fetal thymocytes, whereas there were no positive cells in fetal liver and adult thymus. No positive cells were observed in the spleen, lymph node, or bone marrow. The absorption study, testing for residual cytotoxicity of M6 antibody against GRSL cells, confirmed the above results and showed that the antibody did not react with the nonlymphoid tissues liver, kidney, and brain. Therefore, the antigen detected by M6 antibody was provisionally called fetal thymus antigen-1, FT-1.

Ontogeny of FT-1 Antigen. Strain distribution studies showed that anti-FT-1 (M6 antibody) reacted with the fetal thymocytes of all the inbred mouse strains examined, including BALB/c. As shown in Table II, the proportion of fetal thymocytes positive for FT-1 sharply decreased with increase in gestation time. A small percentage of positive cells still remained in the thymus of neonatal mice, but almost completely disappeared from that of adult mice (7–8 wk). In contrast, Thy-1-positive cells increased in inverse proportion in the fetal thymus. The ontogenic appearance of FT-1 antigen seemed to be almost completely identical to that of DBA lectin receptor as reported previously (7).

FT-1 Antigen on Leukemia Cells. Since anti-FT-1 antibody was raised against GRSL cells, it can be assumed that FT-1 antigen is also expressed on the other leukemia cells. Therefore, a number of mouse leukemias were tested for the expression of FT-1 antigen (Table III) by an immunofluorescence study using biotinylated anti-FT-1 and FITC-avidin. In addition to GRSL cells, cells of 6 out of 14 leukemias tested were brightly stained; ASL-1, RADA-1, and YAC-1 in strain A/J, RL δ -1 in strain BALB/c, and DBA,SL-2 in strain DBA were positive, but AKSL-2, BW5147, and K36 derived from a high leukemic mouse strain, AKR, were all negative.

Biochemical Characterization of FT-1 Antigen. For the estimation of the molecular weight of FT-1 antigen, immunoprecipitation experiments were carried out with NP-40-solubilized antigen from [³H] galactose-labeled GRSL cells, and then the precipitates were run on 7.5% acrylamide SDS gels. The fluorograph shown in Fig. 1 indicates that the apparent molecular weight of FT-1 is ~130,000. A similar result was obtained when immunoprecipitates were derived from cells metabolically labeled by the incorporation of [³⁵S]methionine, although 55,000 and other minor bands were also obtained (Fig. 2). These results clearly indicate that FT-1 antigenic determinants of the leukemic cells reside on the 130,000-dalton glycoproteins. Further biochemical characterization of FT-1 antigen by

TABLE III
Expression of FT-1 Antigen and DBA Receptor on Various Leukemia Cells

Tumor	Host strain	FT-1	DBA receptor
ASL-1	A/J	+	+
RADA-1	A/J	+	+
YAC-1	A/J	+	+
AKSL-2	AKR	-	-
BW5147	AKR	-	-
K36	AKR	-	-
BALB, RV-1	BALB/c	-	-
MOPC-70A	BALB/c	-	-
RL δ -1	BALB/c	+	+
EL-4	C57BL/6	-	-
ERLD	C57BL/6	-	-
DBA, SL-2	DBA	+	+
P815	DBA	-	-
GRSL	GR	+	+

Expression of FT-1 antigens and DBA receptors on various leukemia cells was determined by immunofluorescent staining.

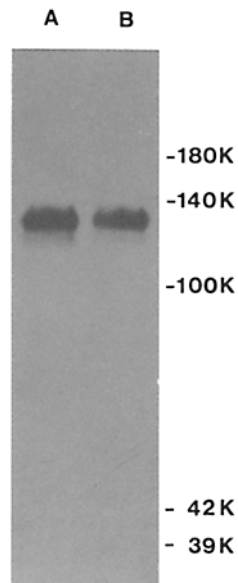


FIGURE 1. SDS-PAGE analysis of the FT-1 antigen immunoprecipitated from [^3H] galactose-labeled GRSL cells. GRSL cells were cultured with [^3H] galactose for 16 h and treated with 0.5% NP-40. The lysates were immunoprecipitated with anti-FT-1 followed by goat anti-mouse IgM, and collected with fixed *S. aureus*. The immunoprecipitates were run on 7.5% polyacrylamide gels under reducing (A) and nonreducing conditions (B). K, $\times 10^3$.

two-dimensional gel electrophoresis confirmed the above results indicating size homogeneity. As shown in Fig. 3, however, the fluorographic pattern of [^3H] galactose-labeled FT-1 antigen shows a family of glycoproteins with extensive charge heterogeneity.

FT-1, A NEW LEUKEMIA ANTIGEN

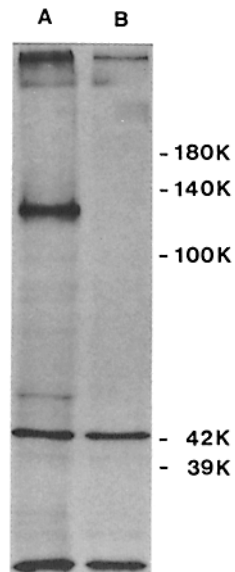


FIGURE 2. SDS-PAGE analysis of the FT-1 antigen immunoprecipitated from [^{35}S] methionine-labeled GRSL cells. GRSL cells were cultured with [^{35}S] methionine for 16 h and treated with 0.5% NP-40. The lysates were immunoprecipitated with anti-FT-1 (A) or normal mouse serum (B), followed by anti-mouse Ig and goat anti-rabbit Ig. The immunoprecipitates were run on 7.5% polyacrylamide gels under reducing conditions.

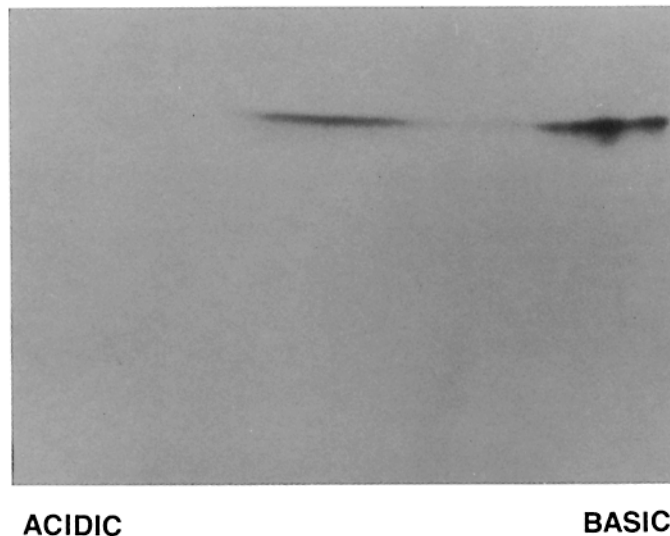


FIGURE 3. Two-dimensional gel electrophoresis of FT-1 antigen immunoprecipitated from [^3H] galactose-labeled GRSL cells. The first dimension separation was by nonequilibrium pH-gradient electrophoresis (basic protein on the right). The second dimension separation (top to bottom) was by SDS-PAGE on 10–16% acrylamide gradient gels.

Relationship between FT-1 Antigen and DBA Receptor. Concerning the ontogeny and the expression of leukemia cells of FT-1 antigen (Tables II and III), there are a number of similarities between FT-1 antigen and DBA lectin receptors. In order to clarify the relationship between these two molecules on GRSL leukemic

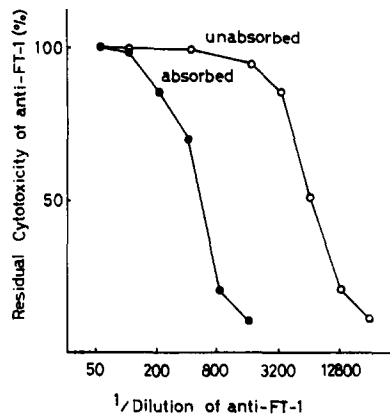


FIGURE 4. Residual cytotoxicity of anti-FT-1 after absorption with DBA receptors. 50 μ l of anti-FT-1 was absorbed with DBA receptors (5 μ g), and then tested for residual cytotoxicity against FT-1⁺ YAC-1 cells.

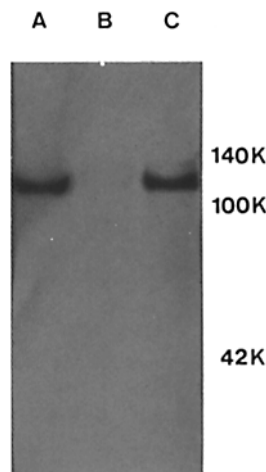


FIGURE 5. Absorption of [³H] galactose-labeled DBA receptor by anti-FT-1 demonstrated by immunoprecipitation. DBA receptors from [³H] galactose labeled GRSL cells were purified by DBA-Sepharose 4 B column and were subjected to SDS-PAGE analysis (A). The DBA receptors were immunoprecipitated either with anti-FT-1 or a control, unrelated IgM hybridoma antibody, anti-FT-2 (Takashi et al., manuscript in preparation), followed by goat anti-mouse IgM, and collected with fixed *S. aureus*. After the precipitate was spun down, the supernatant was subjected to SDS-PAGE analysis. Immunoprecipitation with anti-FT-1 removed the band (B), whereas that with anti-FT-2 did not (C).

cells, the following experiments were carried out. First, GRSL tumor cells were lysed in 0.5% NP-40 extraction medium and the supernatant was applied to a column of DBA Sepharose 4B. 5 μ g of the receptor molecules eluted from the DBA Sepharose 4B column by 0.1 M *N*-acetylgalactosamine was mixed with 50 μ l of anti-FT-1 antibody. After the precipitate was spun down, the supernatant was serially diluted and tested for residual cytotoxicity against YAC-1 cells. Fig. 4 shows that the cytotoxic activity of anti-FT-1 antibody was absorbed with DBA receptor. Second, it was demonstrated that DBA receptors were immunoprecip-

itated by anti-FT-1. Although the molecular weight of [³H]galactose-labeled DBA receptors was slightly lower than that of FT-1 (Fig. 5A), these receptor molecules were specifically absorbed with anti-FT-1 and no precipitation band was observed as shown in Fig. 5B. However, an unrelated IgM hybridoma antibody, anti-FT-2 used as a control, could not absorb DBA receptors so that the precipitation band remained (Fig. 5C). These results strongly suggest that the FT-1 molecule resides on the DBA lectin receptor.

Discussion

The present study demonstrated a unique cell surface antigen designated FT-1, which is expressed on fetal thymocytes of all mouse strains examined, but not on thymocytes or other lymphoid cells of adult mice. This antigen is present also on some leukemia cells, and it is found on the receptor molecules for DBA lectin on GRSL leukemia cells. One of the interesting features of FT-1 antigen is the timing of its expression in connection with thymocyte ontogeny. A majority of fetal thymocytes at 13 d gestation express FT-1 antigen, whereas no positive cells are found in the fetal liver. Therefore, FT-1 antigen seems to appear as soon as the stem cells have reached the thymus. The proportion of FT-1⁺ cells then declined sharply with increase in the time of gestation, while Thy-1⁺ cells increased in inverse proportion. The results suggest that FT-1⁻ stem cells acquire FT-1 antigen, and then differentiate into Thy-1⁺ FT-1⁻ cells under the influence of thymic epithelial cells, although the possibility remains that Thy-1⁺ cells are derived from a minor FT-1⁻ population in the embryonic thymus. In neonatal mice, a small percentage of FT-1⁺ cell still remain in the thymus, but these cells almost completely disappear from the adult thymus, spleen, lymph nodes, and bone marrow. The differentiation of mouse T cells has been extensively studied by use of cell surface antigens (14-16). In the first stage of intrathymic differentiation, stem cells migrate into the cortical area of the thymus, where they acquire Thy-1, TL, and Lyt antigens. These cortical cells, which have Thy-1 antigen in high density, are cortisone-sensitive and relatively large in size. With further maturation, TL antigens are lost and then Thy-1 diminishes in density. Thereafter, they migrate to the thymus medulla where they are medium-sized cells and are resistant to cortisone. Finally they migrate from the thymus medulla to the peripheral lymphoid organs. In contrast to the differentiation sequence within the adult thymus as described above, little is known about the surface antigens of fetal thymocytes before they acquire a number of T cell antigens. Although rabbit anti-asialo GM1 antibody with potent anti-natural killer (NK) activity (17) has been shown to react with fetal thymocytes (18), it is evident that anti-FT-1 marks fetal thymocytes more specifically. Thus, FT-1 antigen will be a useful marker for studying the early stages of T cell ontogeny. Experiments are now in progress to elucidate the mechanism of the acquisition as well as the processing of FT-1 in the thymus of the fetal mouse.

It is frequently said that the presence of embryonic tissue antigen is shared with tumor antigen. However, in lymphoid tissues, such antigens have not ever been serologically defined to date. Since FT-1 is present on fetal thymocytes and some leukemia cells, this is probably the first example of an oncofetal antigen of a mouse T cell leukemia. Therefore, FT-1 antigen will be as important as TL

antigen for understanding the mechanism underlying the activation of normally silent genetic information in leukemic cells.

The molecular weight of FT-1 antigen on leukemic cells was estimated to be 130,000 by means of [³H]galactose labeling followed by immunoprecipitation. FT-1 antigen biosynthetically labeled with [³⁵S] methionine also appeared as a major band with a mol wt 130,000. The other minor bands, especially the 55,000 band, were also brought down. It is possible to assume that 55,000 molecule is an intracellular form of FT-1 antigen that is not detected by sugar labeling. However, further experiments are necessary to study kinetics of FT-1 antigen synthesis in the cells. Two-dimensional gel electrophoresis revealed that FT-1 antigenic determinants appear to reside on a family of glycoproteins with extensive charge heterogeneity. In these biochemical studies, we did not succeed in detecting FT-1 molecules on fetal thymocytes so far attempted, probably because the number of fetal thymocytes used for these experiments was not sufficient. The molecule defined by anti-FT-1 is clearly different from other T cell differentiation antigens in terms of biochemical properties and tissue distribution. Considering the reciprocal appearance of Thy-1⁺ cells and FT-1⁺ cells, it could be possible to assume that the FT-1 molecule is a precursor of other T cell differentiation antigens. Further biochemical and genetic analysis of FT-1 antigen will contribute to understanding of the ontogenic development of T cells as well as leukemogenesis of T cell leukemias.

Summary

A mouse monoclonal antibody (IgM) was obtained by cell hybridization between X63-Ag8.653 myeloma cells and spleen cells from a BALB mouse that was immunized with GRSL leukemia cells of the GR strain. This antibody identified a unique fetal antigen, which is expressed exclusively on embryonic thymocytes of all strains tested. Therefore, the antigen defined was named fetal thymus antigen-1, FT-1. The proportion of FT-1⁺ fetal thymocytes detected by immunofluorescence assay sharply decreases as gestation time increases, and finally they disappear from the thymus. On the other hand, Thy-1⁺ cells increase in inverse proportion. The immunofluorescence studies and absorption tests showed that FT-1 antigen is not detectable on brain, liver, kidney, or lymphoid tissue cells of adult mice. However, it is expressed on some leukemia cells of various mouse strains, which demonstrated that this is the first example of an oncofetal antigen of a mouse leukemia. The molecular weight of FT-1 antigen on leukemia cells was estimated to be 130,000 by means of biosynthetic labeling with [³H]galactose and [³⁵S]methionine. The two-dimensional gel electrophoresis pattern of FT-1 antigen shows a family of glycoproteins with extensive charge heterogeneity. It was also shown that the FT-1 antigen molecule carries the receptor for DBA lectin.

The authors are grateful for the excellent secretarial help of Ms. Noriko Katsu.

Received for publication 12 October 1983 and in revised form 7 December 1983.

References

1. Boyse, E. A., and L. J. Old. 1978. The immunogenetics of differentiation in the mouse. *Harvey Lect.* 71:23.
2. Cantor, H., and E. A. Boyse. 1977. Lymphocytes as models for the study of mammalian cellular differentiation. *Immunol. Rev.* 33:105.
3. Owen, J. J. T., and M. C. Raff. 1970. Studies on the differentiation of thymus-derived lymphocytes. *J. Exp. Med.* 132:1216.
4. Kamarck, M. E., and P. D. Gottlieb. 1977. Expression of thymocyte surface alloantigens in the fetal mouse thymus *in vivo* and in organ culture. *J. Immunol.* 119:407.
5. Ewijk, W. V., E. J. Jenkinson, and J. J. T. Owen. 1982. Detection of Thy-1, T-200, Lyl-1 and Lyl-2-bearing cells in the developing lymphoid organs of the mouse embryo *in vivo* and *in vitro*. *Eur. J. Immunol.* 12:262.
6. Etzler, M. E., and E. A. Kabat. 1970. Purification and characterization of a lectin (plant hemagglutinin) with blood Group A specificity from *Dolichos biflorus*. *Biochemistry.* 9:869.
7. Kasai, M., Y. Ochiai, S. Habu, T. Muramatsu, T. Tokunaga, and K. Okumura. 1980. A new differentiation marker selectively expressed on mouse fetal thymocytes. *Immunol. Lett.* 2:157.
8. Muramatsu, T., H. Muramatsu, M. Kasai, S. Habu, and K. Okumura. 1980. Receptors for *Dolichos biflorus* agglutinin: new cell surface markers on a spontaneous leukemia. *Biochem. Biophys. Res. Commun.* 96:1547.
9. Kearnei, J. H., A. Radruch, B. Liesegang, and K. Rajewsky. 1979. A new mouse myeloma cell line that had lost immunoglobulin expression but permits the construction of antibody-specific hybrid cell lines. *J. Immunol.* 123:1548.
10. Galfre, G., S. C. Howe, C. Milstein, G. W. Butcher, and J. C. Howard. 1978. Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature (Lond.)* 266:550.
11. Takahashi, T., L. J. Old, and E. A. Boyse. 1970. Surface alloantigens of plasma cells. *J. Exp. Med.* 131:1325.
12. Chamberlain, J. P. 1979. Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble fluor, sodium salicylate. *Anal. Biochem.* 98:132.
13. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250:4007.
14. Boyse, E. A., and L. J. Old. 1969. Some aspects of normal and abnormal cell surface genetics. *Annu. Rev. Genet.* 3:269.
15. Raff, M. C. 1971. Surface antigenic markers for distinguishing T and B lymphocytes in mice. *Transplant. Rev.* 6:52.
16. Cantor, H., and I. Weissman. 1976. Development and function of subpopulations of thymocytes and T lymphocytes. *Prog. Allergy.* 20:1.
17. Kasai, M., M. Iwamori, Y. Nagai, K. Okumura, and T. Tada. 1980. A glycolipid on the surface of mouse natural killer cells. *Eur. J. Immunol.* 10:175.
18. Habu, S., M. Kasai, Y. Nagai, N. Tamaoki, T. Tada, L. A. Herzenberg, and K. Okumura. 1980. The glycolipid asialo GM1 as a new differentiation antigen of fetal thymocytes. *J. Immunol.* 125:2284.