## *Brief Definitive Report*

# HIGH INCIDENCE OF B CELL LYMPHOMAS DERIVED FROM THYMECTOMIZED AKR MICE EXPRESSING TL.4 ANTIGEN

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AKR mice show a high incidence of spontaneous lymphomas that are virtually all of the T lymphocyte type and arise predominantly in the thymus (1). Thymus removal at the age of 1–3 mo markedly reduces the spontaneous tumor incidence. These thymectomized mice develop a low incidence of extrathymic lymphoid tumors, which appear late in life. Some lymphomas occurring sporadically in peripheral lymphoid tissues of old intact or thymectomized mice were shown to have a dual nature, bearing characteristics of both T and B cells (2, 3). Although the thymus has been considered as the primary site of neoplastic transformation and proliferation, we have shown previously (4) that premalignant lymphoid cells (PLC) were present in fetal liver of 16-d-old AKR embryos, and among bone marrow (BM) cells of intact AKR mice from the age of 14 d onwards. Thus, using the transplantation bioassay method, we showed (4) that bone marrow cells from AKR mice of different ages, when transferred into intact irradiated (AKR × DBA/2)$F_1$ recipients, caused T cell lymphoma development of AKR origin in 80–100% of the recipients. In contrast, transplantation tests failed to reveal the presence of PLC in the thymus or spleen during the first few months of life (5, 6). These findings suggest that the thymus provides a suitable environment for the proliferation and differentiation of PLC present in BM since birth. PLC present in intact mice have been shown to differ qualitatively from leukemic cells. They have the characteristics of prothymocytes, and require the thymus microenvironment for further proliferation into autonomous leukemic cells (7). Since we suggest that the initial site of PLC occurrence is among bone marrow cells, they should be present irrespective of presence or absence of the thymus.

In this study, we tested whether 8–12-mo-old AKR mice thymectomized at the age of 40–60 d were carriers of dormant PLC that could be triggered to develop into lymphoid tumors after transplantation of BM cells into the appropriate recipients. The developing lymphomas were screened with various T and B cells conventional and monoclonal antibodies (mAb) to define their characteristics.

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## Materials and Methods

*Mice.* AKR/J and (AKR/J × DBA/2)$F_1$ mice were obtained from the breeding center at The Weizmann Institute of Science, and from The Jackson Laboratory (Bar Harbor, ME).

*Monoclonal Antibodies and Antisera.* Mouse anti-mouse Thy-1.1 (HO-22-1) IgM, anti-I-$A^k$ (10-2-16), rat anti-mouse Lyt-1 (53-7.3), and anti-Lyt-2 (53-6.7) were obtained from Salk Institute Distribution, San Diego, CA. Mouse anti-mouse MCF (18-5) IgG2b was a gift from J. Portis, Rocky Mountain Laboratories, MT (8). Rat anti-RA3-6B2 and anti-RA3-2C2 were obtained from R. Coffman (DNAX, Research Institute of Molecular and Cellular Biology Inc., Palo Alto, CA) (9). Rat anti-mouse Fc receptor (FcR) (IgG1) NEI-032 was purchased from New England Nuclear, Boston, MA. Goat anti-mouse $\mu$ chain (anti-38-C-13 IgM) $F(ab)_2$ was a gift from J. Haimovich, Tel Aviv University, Tel Aviv, Israel. Rabbit anti-$\delta$ chain was obtained from I. Zan-Bar, The Weizmann Institute of Science. Goat anti-IgM (heavy and light chains) was purchased from Bio-Yeda, Rehovot, Israel. Anti-TL.4 was prepared in our laboratory, as previously described (6). The fluorescein isothiocyanate (FITC) conjugates of anti-mouse IgG (Meloy Laboratories, Inc., Springfield, VA), anti-rat IgG, anti-goat IgG, anti-rabbit IgG (Bio-Yeda), and anti-mouse Ig (Nordic, Tilburg, The Netherlands) were used for fluorescence labeling.

*Immunofluorescence and Flow Cytometry (FCM).* Immunofluorescence was performed as described in detail elsewhere (6). Fluorescence was measured by FCM analysis, using FACS II (Becton-Dickinson Immunocytochemistry Systems, Mountain View, CA).

## Results

*Identification of PLC Among BM Cells.* The leukemogenic potential of BM cells from thymectomized (when 40–60 d old) AKR mice, removed at age 250–360 d, was tested. BM cells were transplanted into intact or thymectomized, irradiated (400 rad whole-body irradiation) or nonirradiated 6-wk-old AKR or (AKR × DBA/2)$F_1$ mice (presence of thymus and irradiation of the recipient are prerequisites for the development of PLC derived from 14-d-old AKR mice into T cell lymphoma [7]). Hybrid mice were used as recipients to verify the AKR origin of the developing tumors (by serological or transplantation tests). The results obtained, summarized in Table I, clearly indicate that each thymectomized AKR mouse was a carrier of PLC, since we obtained a 100% lymphoma incidence of AKR origin after BM transplantation. The absence of thymus in the recipients and lack of radiation pretreatment did not affect the high incidence of lymphoma development in the BM recipients. All the lymphomas developed extrathymically, predominantly in the spleen, and in many cases, also involved lymph node and liver dissemination.

*Characteristics of Tumors.* The cell surface phenotypes of these obtained

TABLE I

*Leukemogenic Potential of BM from Thymectomized AKR Mice*

| Exp. | Age of BM donors* | Treatment of BM recipients | Leukemia incidence of AKR origin | Latency |
|---|---|---|---|---|
| | *d* | | | *d* |
| 24-172-182 | 300 | $AKD_2$ Thx‡ —30 d→ 400 rad —24 h→ + BM i.v.§ | 11/11 (100%) | 140 ± 10 |
| 24-516-520 | 330 | AKR Thx → + BM i.v. | 5/5 (100%) | 155 ± 22 |
| 24-666-671 | 360 | $AKD_2$ → + BM i.v. | 5/5 (100%) | 35 |
| 25-195-200 | 250 | AKR Thx —20 d→ 400 r —24 h→ + BM i.v. | 4/5 (80%) | 220 ± 12 |

* AKR mice were thymectomized at the age 40 d (Exp. 24-172-182), or 60 d (all other groups).

‡ Thx, thymectomized.

§ BM transfer was done from one donor to one recipient; 3–4 × $10^7$ cells injected intravenously (i.v.).

lymphoid tumors were examined by flow microfluorometry performed on a Becton-Dickinson FACS II. All the 25 lymphomas obtained were found to be Thy-1$^-$, but were positively stained with anti IgM. 12 out of these 25 lymphomas were analyzed more extensively with antibodies that could indicate the T or B cell attributes of these lymphoid tumors: anti-Thy-1, anti-$\mu$, anti-$\delta$, RA3-2C2 (which reacts with pre-B and B cells, and weakly with peripheral T cells [9]), RA3-6B2 (reacts only with B cells [9]), anti-I-A$^k$, and anti-FcR. We also tested the possible expression of the thymus leukemia alloantigen, TL.4, on the cell surface of these lymphomas, because we had previously found (6) its expression on BM cells of 1–15-d-old AKR mice. Since anti-Lyt-1 was shown to mark a subpopulation of B cells at several different stages of differentiation (10), as well as B cell lymphomas (11), we included it in testing the lymphomas. mAb 18-5, which reacts with the envelope recombinant virus, MCF, (8) (considered to have a major role in T cell leukemia development in AKR mice) was also included in the analysis to obtain preliminary evidence of the possible involvement of the AKR recombinant virus in the development of these tumors. The results of these immunofluorescence studies are summarized in Table II, and representative fluorescence profiles are shown in Fig. 1. All tumors were Thy-1$^-$, and stained with antibodies specific for B cells. The expression of Lyt-1 on these B cell lymphomas coincides with similar observations by other investigators (11, 12). The most surprising observation was the expression of TL.4 on all tested tumors.

To ascertain that the surface markers of these lymphomas were integral membrane components of known molecular weight, the following tests were performed: lactoperoxidase iodination (13) to scan for cell surface markers, and metabolic labeling with [$^{35}$S]methionine for the biosynthesis of the relevant markers. A comparative analysis of a T and B cell lymphoma derived from AKR mice was done. The lysates from the tumors were immunoprecipitated with different antibodies and run on sodium dodecyl sulfate–polyacrylamide gel electrophoresis, with suitable markers (14). $\mu$ heavy chain (~68,000 mol wt) was immunoprecipitated from the B cell lymphoma (24–180), and a surface glycoprotein of 220,000 mol wt was immunoprecipitated with mAb RA3-2C2, in

TABLE II

*Cell Surface Phenotypes of Lymphoid Tissues Analyzed on FACS II*

| Tumor | TL.1 | TL.4 | Thy-1 | $\mu$ | $\delta$ | 2C2 | 6B2 | I-A | FcR | Lyt-1 | Lyt-2 | 18-5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24–177 | – | + | – | + | – | + | + | ND | ND | ND | ND | ND |
| 24–178 | – | + | – | + | + | + | + | + | + | + | – | + |
| 24–180 | – | + | – | + | + | + | + | – | + | + | – | + |
| 24–182 | – | + | – | + | – | + | + | + | + | + | – | – |
| 24–516 | – | + | – | + | + | + | + | + | ND | ND | ND | ND |
| 24–666 | – | + | – | + | – | + | + | + | + | + | – | – |
| 24–667 | – | + | – | + | – | + | + | – | + | + | – | – |
| 24–668 | – | + | – | + | + | + | + | + | ND | + | – | – |
| 24–669 | – | + | – | + | + | + | + | + | ND | + | – | + |
| 24–671 | – | + | – | + | – | + | + | – | + | ND | – | – |
| 25–195 | ND | ± | – | + | – | + | + | + | ND | ND | ND | ND |
| 25–197 | ND | ± | – | + | + | + | + | + | + | + | – | ND |

The following antibodies were used: monoclonal anti-Thy-1.1 (clone HO-22-1), anti-TL.1 (clone 65/26), anti-Lyt-1 (clone 53-7·3); anti-Lyt-2 (53.6·7), RA3-2C2[5], RA3-6B2[5], anti-FcR (NEI-032), anti-MCF (18-5)[12], and anti-IA$^k$ (clone 10-2-16); polyclonal anti-TL.4[10], goat anti-$\mu$ (anti-38-C-13 IgM) F(ab')$_2$, and rabbit anti-$\delta$. For staining, the relevant FITC conjugates were used: goat anti-mouse Ig, goat anti-mouse IgG, goat anti-rabbit IgG, rabbit anti-rat IgG, and rabbit anti-goat IgG. (+) indicates ≥40% positive cells, with relative fluorescence intensity of ≥100 U. (–) indicates 0–20% positive cells, with <100 U (see FACS II charts). ND, not done.

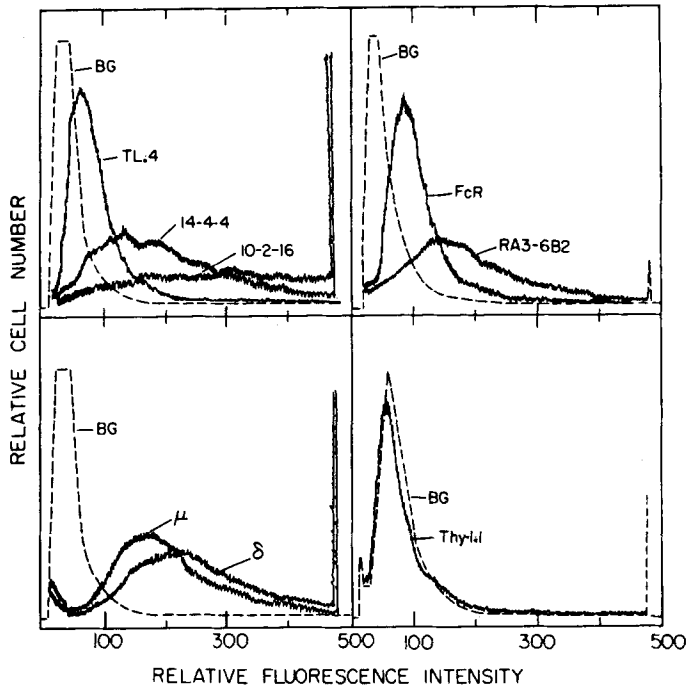


FIGURE 1. Immunofluorescence was performed as described in detail elsewhere (6). FACS II fluorescence intensity profiles of B cell leukemia 24-180 reacted with mouse anti-mouse, anti-TL.4, anti-I-$E^k/C^k$ (clone 14-4-4), anti-I-$A^k$ (clone 10-2-16), anti-Thy-1.1 (clone Ho-22-1), rat anti-mouse FcR (NEI-032), RA3-6B2, rabbit anti-mouse $\delta$ chain, and goat anti-mouse $\mu$ chain, then stained with the relevant FITC conjugate of goat anti-mouse Ig, rabbit anti-rat IgG, rabbit anti-goat IgG, and goat anti-rabbit IgG. BG means background when cells were stained with the second antibody only.

contrast to the T cell lymphoma being negative for both markers. The synthesis of different glycoproteins by a B cell lymphoma and not by the T cell lymphoma tested was demonstrated by immunoprecipitation with anti-IgM and anti-I-A. TL.4 was common for both T and B lymphomas. The anti-IgM antiserum specifically immunoprecipitated a single light chain (~20,000 mol wt) and two heavy chains that correspond to the cytoplasmic (~69,000 mol wt) and membranal (75,000 mol wt) chains. Anti-TL.4 precipitated a glycoprotein of ~48,000 and anti-I-$A^k$ precipitated two chains, $\alpha$ and $\beta$, of ~30,000 mol wt, and a third band, probably the invariant chain.

## Discussion

Herein, we show the capacity of PLC harbored in 250–360-d-old thymectomized AKR mice to induce a high incidence of B cell lymphomas after their transfer into appropriate recipients (4). A high incidence of T cell lymphomas was obtained when PLC from intact AKR mice of similar age were transplanted into the appropriate recipients. Thus, regulatory signals provided by the intact thymus of AKR mice influence the direction of differentiation of the preleukemic pluripotent stem cells. In previous studies (6) we showed an inverse age-related expression of TL.4 on T cell lymphomas, the maximal level (58% $TL.4^+$ tumors) in lymphomas developing in 5–6-mo-old AKR mice. Exposure of young AKR

mice to X-rays rendered most developing T cell lymphomas TL.4$^+$ (6). Our present findings of TL.4$^+$ B cell lymphomas suggest that a common progenitor TL.4$^+$ cell (pluripotential stem cell sharing pre-T and pre-B properties) could serve as a precursor for both T and B cell lymphomas of AKR origin. The fact that all B cell lymphomas tested expressed Lyt-1 and TL.4 antigens, previously believed to be confined to T cells, may also suggest a common T-B cell differentiation pathway. These results may confirm the observations (15) that adult BM contains a population of pluripotent stem cells that can give rise to both B and T lymphocytes.

The involvement of MCF in the development of the B cell neoplasms tested is doubtful, since cells stained with 18-5 were observed only in three out of eight tumors tested (Table II). The possible contribution of ecotropic murine leukemia virus (MuLV) to B cell lymphoma development has been suggested in recent studies by Fredrickson et al. (16). The inbred Swiss strain, NFS, which lacks the genetic information for ecotropic MuLV develops a low incidence of nonthymic lymphomas at the age of 12–18 mo. Insertion of AKR viral genes (Akv-1 and Akv-2) on NFS background (resulting in NFS-V congenic mice for ecotropic MuLV induction loci) rendered them more susceptible (23.8% lymphomas in NFS-V congenic mice vs. 7% in NFS mice) to the development of nonthymic lymphomas of B cell phenotype (16).

## Summary

AKR mice, 6–12 mo after birth, display a high incidence of spontaneous T cell lymphomas that can be prevented by thymus removal at the age of 1–3 mo. We report here the presence of dormant preleukemic cells among bone marrow cells of 8–12-mo-old AKR mice that have been thymectomized when 40–60 d old. Transplantation of bone marrow cells from these thymectomized AKR donors into syngeneic or hybrid (AKR × DBA/2)$F_1$ intact or thymectomized recipients resulted in lymphoma development of AKR origin in 80–100% of the recipients. Analysis, by flow microfluorometry, of the antigenic cell surface phenotypes of the developing lymphomas revealed that all tumors were B cell lymphomas, since the cells stained with class-specific anti-IgM reagents and other reagents specific for B cells (RA3-2C2, RA3-6B2, anti-I-A, and anti–Fc receptor), and were Thy-1$^-$. All these B cell tumors also expressed two T cell differentiation antigens, TL.4, found exclusively on T cell lymphomas, and Lyt-1 antigen, previously shown (11) to be expressed on some B cell neoplasms. The surface markers $\mu$, I-A, RA3-2C2, and TL.4 identified by immunofluorescence, were shown to be integral membrane components synthesized by the tumor cells, rather than passively acquired proteins.

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