Brief Definitive Report

LACK OF GENE REARRANGEMENT AND mRNA EXPRESSION OF THE BETA CHAIN OF THE T CELL RECEPTOR IN SPONTANEOUS RAT LARGE GRANULAR LYMPHOCYTE LEUKEMIA LINES

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In the rat (1), mouse (2), and human (3), most, if not all, natural killer (NK) activity has been shown to be associated with a distinct subpopulation of cells termed large granular lymphocytes (LGL). It has been suggested that, since LGL express some T cell–associated antigens (4) and can grow in vitro in cultures supplemented with interleukin 2 (IL-2) (5) that LGL represent a population of lymphocytes within the T cell lineage. At present, however, there is very little definitive information regarding either the lineage of these cells or the nature of their cell surface receptors for antigens. To further address the relationship between LGL and T cells, we examined several transplantable LGL leukemia lines for the rearrangement and expression of the genes encoding for the beta chain of the T cell antigen receptor. This receptor has been shown (6) to be expressed in most, if not all, helper and cytotoxic T cells, but not in suppressor T cells. LGL leukemia lines were studied because they provide a convenient source of highly active NK cells with morphological and functional characteristics that closely resemble those of normal LGL (7, 8). Previous data (7, 8) also suggest that these tumor cell lines represent the clonal expansion of normal LGL, and, presuming an analogy to cytotoxic T cells, would be expected to demonstrate a unique rearrangement of the various elements of the antigen receptor, possibly corresponding to the genes coding for the T cell antigen receptor.

The gene encoding for the beta chain of the T cell antigen receptor has recently been cloned and characterized in both the mouse (9) and human (10). Prerequisites to beta chain gene expression are rearrangements of the variable (V), diversity (D), and joining (J) elements into a transcriptional unit that is completed by the coding exons of the constant (C) region (9). The absence of such rearrangements indicates that a particular cell line does not functionally express the beta chain gene. The rearrangement of this gene is consistent with, but does not prove, transcription and translation of functional messenger RNA (mRNA) or receptor expression. However, the presence of a 1.2–1.3 kilobase

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(kb) mRNA hybridizing to the beta chain probe would indicate functional transcription of the rearranged beta chain gene locus. The present study uses the beta chain cDNA clone, 86T5, to examine four rat LGL leukemia lines for the expression of unique rearrangements and mRNA transcription of the beta chain for the T cell antigen receptor.

Materials and Methods

Cell Lines. The rat LGL leukemia lines used in this study (RNK-0, RNK-4, RNK-8, and RNK-10) were derived from spontaneous tumors that arose in aged Fischer (F344) rats (7, 8). The four lines used in this study were serially passaged in ascites and shown to have very high levels of both NK and antibody-dependent cell-mediated cytotoxicity (ADCC) activities. Passage numbers are indicated in the text (e.g., RNK-0 passage 14 ascites is designated as RNK-0p14A). With the exception of their neoplastic nature, the characteristics of these cell lines have been shown to be very similar to normal LGL isolated from the peripheral blood of F344 rats (7, 8). The G-1 and (C58NT)D cell lines were derived from Gross virus–induced lymphomas in WF/N rats and maintained in vitro in RPMI 1640 plus 5% fetal bovine serum. Seven Moloney leukemia virus–induced T cell lymphomas in F344 rats were obtained, after serial transplantation in vivo, from Dr. David Steffen (Worcester Foundation for Experimental Biology, Worcester, MA). The murine cell line C10 is an in vitro grown T cell hybridoma that produces IL-2 in the presence of hen egg lysozyme antigen.

Preparation of DNA and RNA. High molecular weight DNA was prepared by the method of Blin and Stafford (11) as previously described (9). Cytoplasmic RNA was prepared by Nonidet P-40 lysis of the plasma membrane, pelleting of nuclei at 2,000 *g*, and phenol extraction of cytoplasmic RNA in the presence of sodium dodecyl sulfate (SDS), EDTA, and urea (12).

Southern Blots. DNA was digested with a 10-fold excess of PvuII or EcoRI, ethanol precipitated, and electrophoresed under standard conditions. Gels were denatured, neutralized, and blotted according to Southern (13). Nitrocellulose filters were hybridized with the cDNA insert from clone 86T5, nick translated to a specific activity of $2–4 \times 10^8$ cpm/μg. 20 ml of probe was added to each blot, at 1×10^6 cpm/ml, in 50% formamide, 5× SSPE, 1× Denhardt's, 10% dextran sulfate, 0.1% SDS, and 100 μg/ml denatured salmon sperm DNA. Blots were incubated for 16 h and washed in progressively more stringent conditions, ending with 0.2× standard sodium citrate (SSC) at 55°C.

Northern Blots. RNA (5 μg poly(A)-selected) was electrophoresed in formaldehyde gels under standard conditions. Gels were soaked 1 h in high salt (20× SSC), blotted, and hybridized as above (9).

Results and Discussion

To determine whether the beta chain of the T cell receptor heterodimer is expressed in LGL and might thereby comprise part of the recognition unit expressed by NK cells, beta chain message expression was examined in LGL tumor cells known to exhibit high NK activity. Fig. 1 depicts an autoradiograph of a Northern blot containing RNA from two rat NK lines, RNK-8 and RNK-16 (lanes *3* and *4*), and, as controls, a rat T cell lymphoma line, G-1 (lane *1*) and a murine T cell hybridoma, C10 (lane *2*). To compare relative amounts of mRNA expression, the same blot is shown at two different exposure times: 2 and 16 h. Three points can be made concerning the data from this experiment. First, it is clear that the mouse probe for the beta chain can be used to detect beta chain mRNA from a rat T cell line (G-1). However, in contrast to the mouse, this probe detects equal amounts of 1.0 and 1.3 kb mRNA in the rat T cell line. Second, the intensity of the bands on the autoradiograph indicates that the level of total beta chain message in the two mouse and the rat cell lines is at

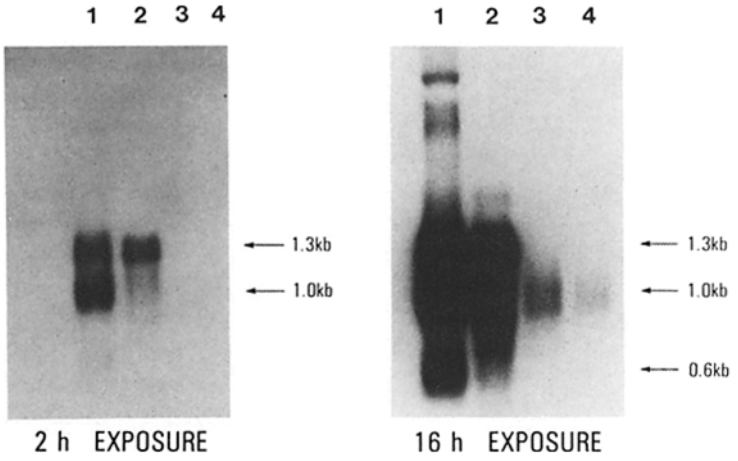

FIGURE 1. Northern blot autoradiograph of mRNA from various rat and mouse cell lines hybridized with radiolabeled cDNA insert from murine beta chain of the T cell receptor probe. (*1*) W/FN rat T cell lymphoma (G-1); (*2*) murine T cell hybridoma (C10); (*3–4*) F344 LGL leukemia lines (RNK-8 and RNK-16, respectively). Two exposures are shown, 2 h and 16 h.

least 20–40 times greater than either of the NK lines. Since the level of message in C10 has been independently estimated to be ~0.05% of the total mRNA, we would estimate the message expression in the LGL lines at <0.002%, or <10 copies per cell. Third, and more important, the size of the mRNA from the LGL leukemias is restricted to the 1.0 kb size. A beta chain message of this size is probably transcribed from an unrearranged or simple DJ rearrangement. It is therefore unlikely that this 1.0 kb message is translated to a functional molecule since messages of similar size have been previously shown in the mouse not to contain a variable region gene element nor a methionyl start codon (14). In contrast, the functionally translatable beta chain mRNA has a size of 1.2–1.3 kb. Based on the density of the 1.3 kb band from the LGL leukemia, if there is a message in the LGL of 1,300 nucleotides, it would have to be expressed at less than one copy per cell. We conclude that these LGL leukemia cells, with high levels of NK activity, do not express normal functional mRNA for the beta chain of the T cell antigen receptor.

Productive expression of the beta chain genes requires gene rearrangements to juxtapose V, D, J, and C regions. To determine whether the rat LGL leukemia lines exhibit beta chain gene rearrangements, DNA samples from four F344 LGL leukemia lines and, as controls, F344 kidney cells and two WF/N rat T cell lines, were digested with EcoRI and PvuII and examined for patterns of hybridizing bands on genomic Southern blots. The pattern of fragments after EcoRI digestion of kidney DNA showed a germline pattern of hybridization with three bands, at 2.9, 3.5, and 6.7 kb (Fig. 2*A*). An identical pattern was seen with all four LGL lines. In contrast, the rat T cell line G-1 gave a distinct pattern of hybridization. Similarly, unique hybridization patterns were seen with one other WF/N T cell line [(C58NT)D] and seven Moloney leukemia virus–induced T cell lymphomas from F344 rats (data not shown), demonstrating that the differences in EcoRI hybridization patterns were not due to a restriction polymorphism in the rat.

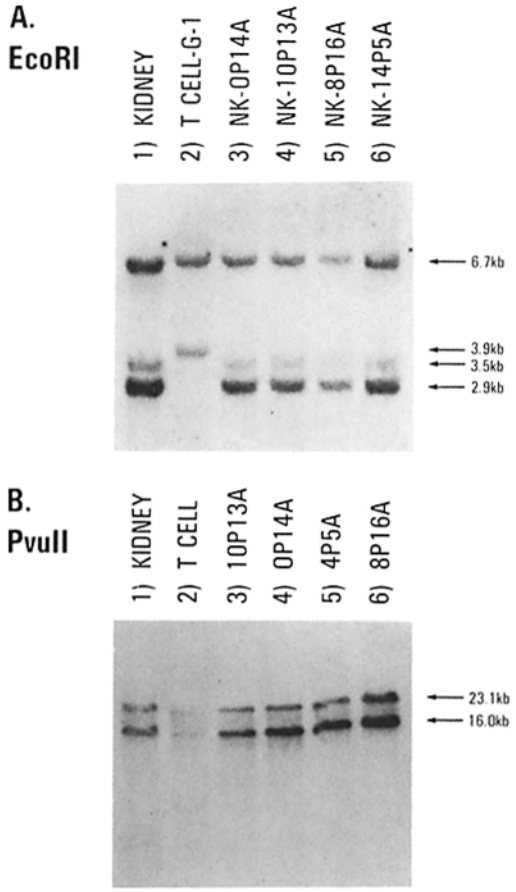

FIGURE 2. Genomic Southern blots of various rat and mouse DNA samples digested with either EcoRI (*A*) or PvuII (*B*). (*1*) F344 rat kidney cells; (2*A*) W/FN rat T cell line (G-1); (2*B*) F344 rat peripheral blood T cells; (*3–6*) F344 LGL leukemia lines.

In the PvuII digests (Fig. 2*B*), the germline pattern was also seen on both LGL leukemia and kidney DNA, as two very large hybridizing bands (16 and 20 kb). Assuming an analogy to the mouse gene (15), the closely spaced C regions (within 6 kb) would mean that at least one of the bands must include both the C and J regions, and that, therefore, any gene rearrangements would be detected at this locus. Such rearrangements were not observed with the LGL leukemias (Fig. 2*B*) but were observed in the F344 T cell tumors (data not shown). The lane labeled T cells in the PvuII digest (Fig. 2*B*) contained DNA from a heterogeneous population of uncloned F344 peripheral blood rat T cells, for which no unique gene rearrangements in the beta chain locus were seen (nor were expected). Similarly, in blots of HindIII and BamHI digests, no gene rearrangements were detected from the LGL leukemias (data not shown). The likelihood of not detecting gene rearrangements in the beta chain locus with these four DNA digests is very small.

The overwhelming conclusion is that rat LGL leukemia lines, which mediate both NK and ADCC activities, do not express a mature or functional form of the beta chain of the T cell receptor. The mRNA expression is solely represented

by aberrant (1.0 kb) transcripts produced at a level <1/20th of that seen in rat or mouse T cell tumors or antigen-specific hybridomas. Furthermore, no gene rearrangements were detected by four different restriction enzymes. Since EcoRI and PvuII digests of two WF/N T cell lines, normal F344 T cells, and seven T cell lines from F344 rats demonstrated unique hybridization patterns, the lack of unique hybridization patterns in the F344 LGL leukemia cells were not due to a restriction polymorphism in the F344 strain.

The present studies have used the LGL leukemia lines because these cells can be obtained in large numbers and in very high purity (>95%), are morphologically identical to normal rat LGL (7), and are highly cytotoxic for NK-susceptible targets but do not kill NK-resistant tumor cells (8). The results with these cells, however, are in marked contrast to those obtained in previous studies in the mouse (16) and human (17), which have suggested that a T cell receptor–like molecule is present in IL-2-propagated cloned cells with NK-like activity. Several possible reasons for this difference are: (*a*) differences between in vitro grown clones derived from normal cells and in vivo derived tumors, (*b*) differences between species, or, most likely, (*c*) differences in the origin of the cytotoxic cells. Since these previous studies (16, 17) examined cytotoxic lines with T cell–like as well as NK-like characteristics, it is quite possible that these lines were derived from T cells rather than LGL. Several groups (16, 17) have reported that clones of cytotoxic T lymphocytes (CTL) can, under appropriate conditions, develop NK-like activity, either together with CTL activity or after the loss of specific reactivity. The finding of T cell receptors on such CTL-derived clones would not be surprising; however, this might not be reflective of the receptor expression on in vivo derived LGL. In fact, since the rat LGL leukemia cell lines that we used maintain a pattern of target cell specificity very similar to normal LGL (8), the present data strongly suggest that NK target cell recognition does not require the expression of the antigen-specific receptor of helper and cytotoxic T cells.

Summary

Using the murine cDNA clone for the beta chain of the T cell antigen receptor, we have examined four highly cytotoxic rat large granular lymphocyte (LGL) leukemia lines for the expression of unique rearrangements and mRNA transcription of the genes coding for the T cell antigen receptor. In contrast to normal rat T cells and nine rat T cell lines, the LGL leukemia lines exhibited no detectable gene rearrangements in the beta chain locus after digestion of LGL DNA by four restriction enzymes. Northern blots containing RNA from these LGL tumor lines demonstrated a low level of aberrant or nonrearranged beta chain transcription (<10 copies per cell) but virtually no translatable 1.3 kilobase message. These results demonstrate that LGL leukemia lines which mediate both natural killer (NK) and antibody-dependent cell-mediated cytotoxicity (ADCC) activities do not express the beta chain of the T cell receptor. The nature of the NK cell receptor for antigen remains elusive.

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