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Immunology

The nonobese diabetic *scid* mouse: Model for spontaneous thymomagenesis associated with immunodeficiency

(severe combined immunodeficiency mutation)

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ABSTRACT **Homozygosity for the severe combined immunodeficiency (*scid*) mutation results in a block in T- and B-lymphocyte development. An unusually high incidence of spontaneous thymic lymphoma development was observed after transfer of this mutation from the C.B-17 congenic strain background onto the diabetes-susceptible nonobese diabetic (NOD) background. Thymomagenesis in the NOD-*scid*/*scid* mouse was associated with expression of an NOD mouse-unique endogenous ecotropic murine leukemia provirus locus (*Emv-30*, mapped to proximal region of chromosome 11) not expressed in the standard substrain NOD/Lt thymus. All tumors exhibited insertions of ecotropic proviruses, whereas only a subset also exhibited proviral integrations of mink cell focus-forming retrovirus. Neither class of retrovirus was associated with consistent integration into genes previously associated with activation of oncogenesis. We propose that the unusual features of T-cell ontogeny characteristic of the NOD inbred strain synergize with the *scid*-imparted block in thymocyte development, leading to activation of the NOD-unique *Emv-30* to initiate thymomagenesis.**

T lymphocyte-mediated destruction of the insulin-producing pancreatic β cells plays a key role in the pathogenesis of spontaneous autoimmune diabetes in the nonobese diabetic (NOD) mouse strain, a model for insulin-dependent diabetes mellitus (1). Diabetes can be adoptively transferred by either T lymphocytes or bone marrow (2, 3). In addition to several abnormal T-lymphocyte functions (4, 5), a dominantly inherited accumulation of T cells in peripheral lymphoid tissues (6) and a defect in thymic maturation (7) have been associated with diabetogenesis in the NOD/Lt mouse substrain.

Studies designed to delineate the separate diabetogenic functions of $CD4^+$ and $CD8^+$ T-cell subsets in adoptive transfer experiments generally have required irradiation of the recipient to deplete endogenous T-cell immunity. Using a different approach to eliminate endogenous T-cell function, we have developed a NOD strain congenic for the severe combined immunodeficiency (*scid*) mutation. Homozygotes for this recessive mutation lack functional T and B lymphocytes (8); thus, NOD-*scid*/*scid* mice are diabetes-resistant (9). During the establishment of this congenic strain, the diabetes-resistant NOD-*scid*/*scid* homozygous mice were distinguished from their nonimmunodeficient sibs (either +/+ or +/*scid*) by an unusually high frequency of spontaneous thymic lymphoma (thymoma) development in the former. This was especially noteworthy because thymic lymphomas have not been detected in intact NOD/Lt-+/+ mice that do not develop diabetes, although such longer-lived NOD/Lt mice in our colony are susceptible to development of a variety of other neoplasms, including nonthymic lymphomas (10).

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Molecular genetic analysis of lymphomagenesis in various inbred strains indicates a causal association between high spontaneous incidence of hematopoietic neoplasms (including thymic lymphomas) and activation of endogenous murine leukemia viruses (MuLVs) (11). The early expression and replication of endogenous ecotropic MuLVs, coupled with somatic integrations of ecotropic or novel recombinant oncogenic proviruses in the genome of target cells and insertional activation of specific cellular protooncogenes, represented common characteristics regardless of the cell type from which these tumors originated (T-, B-, or myeloid-cell lineage) (11, 12).

The objective of the present study was to determine the cellular origin and ontogeny of thymomagenesis in NOD-*scid*/*scid* mice and to evaluate the potential retroviral contribution to the development of these neoplasms.

MATERIAL AND METHODS

Mice. The NOD/Lt colony has been described (13). The *scid* mutation occurred originally in the C.B-17 inbred strain (8). Following an initial C.B-17-*scid*/*scid* × NOD/Lt outcross, the *scid* mutation was transferred onto the NOD genetic background by repeated backcross cycles (N7–N10) of tested +/*scid* heterozygotes selected after each backcross cycle. Mice were maintained under specific pathogen-free conditions, and *scid*/*scid* mice received a prophylactic dose of a sulfamethoxazole/trimetoprim mixture (Sulfatrim; Barre-National, Baltimore) in drinking water for 3 days per week as described (14). For genetic mapping studies, DNA was prepared from an (NOD/Lt × NON.D-*H-2*^{g7})F_2 segregating population provided by L. Wicker and L. Peterson (Merck).

DNA Analysis. High molecular weight DNA was prepared from fresh or frozen tissues by a modification of the method as described (15). After two extractions with 1:1 (vol/vol) phenol/chloroform, DNA was collected by precipitation with ethanol. For Southern blot analysis, DNA samples (10 μg per lane) were digested overnight with appropriate restriction endonucleases under the conditions recommended by the manufacturer (GIBCO/BRL). Digested DNA was separated by electrophoresis in 0.7% agarose gels, transferred to a nylon membrane (Zetabind; Cuno) in 10× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate), and crosslinked to the filter by using UV-Stratalinker (Stratagene). Probe labeling, hybridization conditions, and autoradiography for 1–3 days were as described (13). The final two posthybridization washes were performed at 65°C in 0.1× SSC/0.1% SDS for 30 min each.

Abbreviations: NOD, nonobese diabetic; NON, nonobese nondiabetic; *scid*, severe combined immunodeficiency mutation; MuLV, murine leukemia virus; MCF, mink cell focus-forming retrovirus; FITC, fluorescein isothiocyanate; IL-2 and IL-3, interleukins 2 and 3; TCR, T-cell receptor.

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RNA Analysis. Total RNA was isolated from fresh tissue by a single-step guanidinium isothiocyanate extraction method (16). RNA analysis (20 μg per lane) by electrophoresis in a 1.25% agarose/7% formaldehyde gel, transfer to Zetabind with 20× SSC, and probe hybridizations were as described (17).

Hybridization Probes. Recombinant DNA probes for the ecotropic murine leukemia proviral envelope gene (clone pEco), for the T-cell receptor (TCR) β-chain locus *Tcrb* (clone pUCJ1), and the immunoglobulin heavy-chain locus *Igh* (clone JH) have been described (11) and were provided by H. Bedigian (The Jackson Laboratory). The *Pim-1* genomic probe [probe A (18)] was provided by J. Nadeau (The Jackson Laboratory). A genomic probe for *Myc* (pSVcmyc1) was obtained from the American Type Culture Collection (ATCC no. 41029). A 100-base-pair (bp) *Bst*NI–*Xma* I subclone of the mink cell focus-forming (MCF) viral envelope gene (19) and a chicken β-actin cDNA clone were provided by H. G. Bedigian (The Jackson Laboratory).

Purified inserts were labeled with [α-^{32}P]dCTP by using a random oligonucleotide-labeling kit (Pharmacia).

Tumor Morphology. Sections (5 μm) of paraffin-embedded tissue samples were examined by light microscopy after staining with hematoxylin and eosin. For electron microscopy, a suspension of thymoblastoid cells was prepared from a thymic lymphoma from a 6-month-old NOD-*scid*/*scid* mouse. After passaging as ascites in young NOD-*scid*/*scid* mice, thymic lymphoma cells were harvested and cultured in RPMI 1640 medium containing 15% fetal bovine serum for 7 wk. A cell pellet was processed for ultrastructural examination as described (20) after fixation in 2% glutaraldehyde/1% paraformaldehyde/0.1 M sodium cacodylate, pH 7.2.

Tumor Phenotyping. Thymomas were excised, and single-cell suspensions were prepared by extrusion of cells through Nitex 110 mesh bags. Aliquots containing 10^6 cells were analyzed by flow cytometry with a Becton Dickinson FACScan after staining with the following monoclonal antibodies: biotinylated HO 13-4.9 (anti-Thy-1^b) diluted 1:10, followed by fluorescein isothiocyanate (FITC)-conjugated avidin diluted 1:10; phycoerythrin-conjugated GK1.5 (anti-CD4) diluted 1:5; FITC-conjugated 53-6.72 (anti-CD8) diluted 1:5; Y-CD3-1 (anti-CD3; 50 μl of cell culture supernatant) followed by FITC-conjugated goat anti-rat immunoglobulins (1:10); FITC-conjugated 7D4 [anti-mouse interleukin 2 (IL-2) receptor] diluted 1:30. B lymphocytes and pre-B-cells were detected by using directly FITC-conjugated RA3-6B2 (anti-B220, diluted 1:32).

RESULTS

Incidence, Pathology, and Phenotype of Thymic Lymphomas in NOD-*scid*/*scid* Mice. Cumulative thymic lymphoma incidence was determined in 36 40-wk-old diabetes-free NOD-*scid*/*scid* mice (18 males, 18 females). Most tumors became grossly manifest after 20 wk of age, and the combined incidence at 40 wk in both sexes was 67% (24 of 36 mice; Table 1). A higher tumor frequency was observed in females (83%, 15 of 18) than in males (50%, 9 of 18; Table 1), indicating a tendency for a gender-dependent differential tumor susceptibility in this congenic strain. At autopsy, the size of thymic tumors varied between 200 and 700 mg, and neoplastic enlargements of spleen and lymph nodes (axillary, cervical, mediastinal, and/or mesenteric) were also observed in some individuals. Data for the standard NOD/Lt substrain were not provided in Table 1 because >80% of females and >40% of males in our colony have succumbed to diabetes by 28 wk of age. Thymic lymphomas have not been found in any of the NOD/Lt nondiabetic mice autopsied between 40 and 78 wk of age (10). Thus, the increased incidence of thymomagenesis in the NOD-*scid*/*scid* congenic mouse represents a specific interaction between mutation at the *scid* locus on chromosome 16 and the oncogenic potential latent in the NOD genetic background.

Table 1. Cumulative thymic lymphoma incidence in 40-wk-old NOD-*scid*/*scid* mice (N7F5)

	NOD-*scid*/*scid* mice, no.		
	Total	With tumor	% incidence
Females	18	15*	83
Males	18	9*	50
Combined	36	24	67

*Difference in male and female incidence is not significant by 2 × 2 χ^2 table analysis ($P > 0.3$).

In contrast to NOD/Lt-+/+ or NOD-+/*scid* mice, thymic tissue of NOD-*scid*/*scid* mice lacked a defineable cortex, with the medullary region containing macrophages, epithelial cells, fibroblasts, and few lymphoid cells. All histologically examined tumors consisted of undifferentiated lymphoblastoid cells showing increased mitotic activity. Five of seven independent tumors analyzed for cell-surface T-lymphocyte markers by dual-color fluorescence-activated cell sorter (FACS) analysis were Thy-1.2$^+$, CD3$^-$, CD4$^+$, CD8$^+$. The remaining two were Thy-1.2$^+$, CD3$^-$, CD4$^-$, CD8$^+$. In addition, FACS analysis of two tumors for the IL-2 receptor did not detect cell-surface expression of this antigen. These phenotypes permitted classification of the thymic lymphomas as pre-T-cell lymphoblastic neoplasias. Tumor classification was further expanded by DNA typing of rearrangements within the *Tcrb* and *Igh* loci. These structural genomic changes occur early during T- and B-cell differentiation in normal (i.e., immunocompetent) animals and in *scid*/*scid* mice [although in a nonproductive fashion (21)] and can serve as helpful diagnostic molecular markers for determining the cell lineage of lymphomas and leukemias (22). In the mouse, rearrangement of TCR genes is diagnostic for lymphoid tumors of T-cell origin, whereas *Igh* gene rearrangements are generally diagnostic for lymphoid tumors of B-cell origin, although rearrangements at this locus can also occasionally be found in T-cell lymphomas (11). Southern blot analysis of nine independent NOD-*scid*/*scid* thymic lymphomas revealed rearrangement of *Tcrb* in eight tumors and rearrangement of *Igh* in only one tumor (not shown), providing additional evidence for a T-lineage origin of these neoplasms.

Genetics and Expression of *Emv-30*, the Single Endogenous Ecotropic Provirus in NOD/Lt and NOD-*scid*/*scid* Mice. Previous studies have shown that inheritance and early expression of endogenous ecotropic MuLVs are a prerequisite for the development of lymphomas in susceptible strains (11, 12). We have reported that the NOD/Lt strain and the closely related nonobese normal strain NON/Lt share a single novel ecotropic murine leukemia proviral locus designated *Emv-30* (23). Initial data provided evidence for a linkage of *Emv-30* with *Mpmv-18* (modified polytropic MuLV-18) and *Il-3* [gene encoding interleukin 3 (IL-3)] markers on the proximal part of chromosome 11 (23). Segregation analysis of F_2 data (F_1: NON.NOD-*H-2*^{g7} × NOD/Lt) obtained in this study combined with our previously published BC1 data (23) established the position of *Emv-30* between *Mpmv-18* and *Il-3*. The map positions of *Mpmv-18* and *Il-3* (distances from the centromere) in Fig. 1 are from the July 1991 "Locus map of the mouse" prepared from GBASE.[†] Southern blot analysis of kidney DNA revealed that the only germ line-transmitted ecotropic proviral locus in the genome

[†]Davisson, M. T., Doolittle, D. P., Hillyard, A. L., Guidi, J. N. & Roderick, T. H. (1991) GBASE, On-line Genomic Database for the Mouse (The Jackson Laboratory, Bar Harbor, ME).

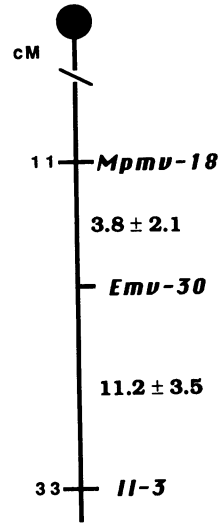

FIG. 1. Position of *Emv-30* on chromosome 11. The position of *Emv-30* on chromosome 11 was determined by combined recombination analysis in a three-point cross involving *Mpmv-18* and *Il-3* markers. A total of 78 informative meioses were analyzed, including data obtained with 36 first backcross progeny [(NOD × NON)F_1 × NOD] (23) and 21 progeny from an F_2 cross [(NON.NOD-*H-2*^{g7} × NOD)F_1 × F_1]. The recombination frequencies represent a weighted average using the information function (24).

of NOD-*scid/scid* mice is, as in standard NOD/Lt and NON/Lt, *Emv-30*.

Ontogeny of *Emv-30* expression was determined by Northern blot analysis of total RNA from thymus and spleen of NOD-*scid/scid*, NOD/Lt, and NON/Lt mice. High levels of full-length [8.4 kilobases (kb)] and *env* (3.0-kb envelope) *Emv-30* transcripts were detected only in NOD-*scid/scid* tissues at 21 days of age (Fig. 2). No *Emv-30* expression was detected either in standard NOD/Lt-+/+ mice at any age or in NOD-*scid/scid* thymus and spleen of 18-day-old fetuses, newborns, and 7-day-old pups (Fig. 2; also data not shown). After an extended exposure (14 days) of the autoradiogram shown in Fig. 2, low levels of Emv-30 transcripts were also detected in NON/Lt thymus and spleen as reported (23). Thus, early, high-level *Emv-30* expression preceding tumor

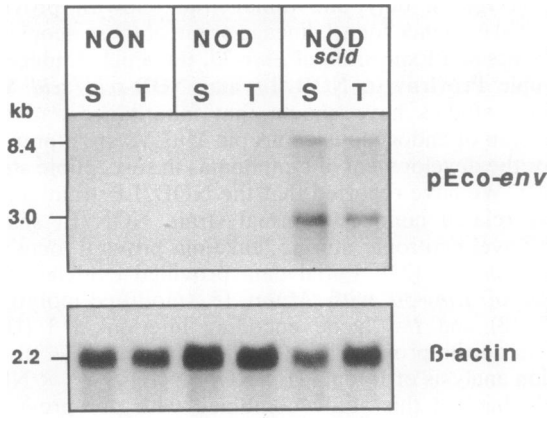

FIG. 2. Northern blot analysis of *Emv-30* expression in strains carrying this provirus. Northern blot of total RNA from spleen (lanes S) and thymus (lanes T) of 21-day-old NON/Lt, NOD/Lt, and NOD-*scid/scid* mice was prepared as described in text. (*Upper*) Blot hybridized with ^{32}P-labeled pEco-*env* probe. Autoradiography was performed for 2 days at −70°C with an intensifying screen. (*Lower*) Same blot rehybridized (after erasing the pEco signal) with a ^{32}P-labeled β-actin probe to demonstrate equal loading of RNA amount in each lane. Autoradiography was performed for 5 hr. Transcript sizes are indicated on the left margin.

manifestation was a characteristic restricted to the thymic lymphoma-prone NOD-*scid/scid* mice and was not present in thymic lymphoma-resistant NOD/Lt-+/+ mice. Thymic expressibility of the *Emv-30* locus in NOD-*scid/scid* mice was consistent with the electron microscopic observation of numerous cell-surface budding and mature type C retroviral particles on thymic lymphoma cells. In contrast, type C retroviruses were not detectable by electron microscopy in uncultured NOD/Lt-+/+ thymocytes.

Somatically Acquired Proviruses, Protooncogene Rearrangement, and Clonal Origin of Thymic Lymphomas. Southern blot analysis of thymic lymphoma DNAs digested with appropriate restriction endonucleases, producing unique proviral DNA–cellular DNA junction fragments, identified genomic fragments indicative of somatically acquired proviral elements. Because of the high *Emv-30* expression in thymus and spleen of NOD-*scid/scid* mice, we initially searched for new proviral integrations using the ecotropic *env*-specific probe pEco on blots of *Xba* I- or *Pvu* II-digested thymic lymphoma DNAs. Because *Xba* I and *Pvu* II, respectively, produce unique 5′ and 3′ ecotropic proviral DNA–cellular DNA junction fragments detected with pEco (25), each fragment should correspond to an acquired ecotropic proviral element. Kidney DNA was used as a control to distinguish somatically acquired proviruses from endogenous *Emv-30*. Kidney DNA exhibited a single 15-kb *Xba* I fragment (Fig. 3 *Upper*) and a 3.7-kb *Pvu* II fragment (Fig. 3 *Lower*). DNA analysis of 18 independent thymic lymphomas revealed in each sample one or multiple new pEco-hybridizing, tumor-specific fragments presumed to mark individual acquired proviruses (Fig. 3). We also analyzed tumor DNAs with the restriction endonuclease *Pst* I, which cleaves once within the long terminal repeats of the ecotropic proviral genome. *Pst* I digestion of genomic DNA generates a single 8.2-kb internal fragment detected with pEco, which is characteristic for the most endogenous *Emv* loci including *Emv-30* (23, 26). When digested with *Pst* I, most tumor DNAs, including the thymic lymphomas shown in Fig. 3 *Upper*, exhibited only a single pEco-hybridizing 8.2-kb fragment (data not shown). This result strongly indicated that the acquired proviruses were full-length ecotropic elements originally encoded by *Emv-30*.

Spontaneous thymic lymphomas and T-cell lymphomas with retroviral etiology in mice are usually caused by oncogenic MCF retroviruses generated *de novo* by recombination between endogenous ecotropic and nonecotropic retroviral sequences in affected individuals (12). Therefore, this class was also analyzed in the same thymic lymphoma DNA panel. DNA digestion with *Eco*RI produces 5′ proviral DNA–cellular DNA junction fragments detectable with the MCF-*env* probe, which does not cross-hybridize with ecotropic *env* (19). In contrast to the presence of new pEco-hybridizing fragments in all 18 examined tumors, new weakly hybridizing MCF fragments were detected in *Eco*RI-digested DNA from only 7 of them. These findings show a consistent association between tumorigenesis and ecotropic, but not MCF proviral, integrations in NOD-*scid/scid* mice.

Although in most affected NOD-*scid/scid* mice the only observed tumor was a thymic lymphoma, multiple lymphomas in lymph nodes and spleen in addition to the thymus were observed in a few individuals. The origin of these multiple neoplasms was analyzed by a comparison of proviral integration patterns of affected tissues in the same individual. In two animals (numbers 16 and 24) with multiple tumors, identical acquired proviral fragments were shared between all tumor tissues of the same mouse (Fig. 3 *Lower*). The finding of shared proviral profile in neoplasms at multiple anatomical sites in the same individual, coupled with the histopathological similarity of the neoplasms, strongly indicated the thymus to be the primary site of tumor development followed by metastatic dissemination. The additional single or multiple

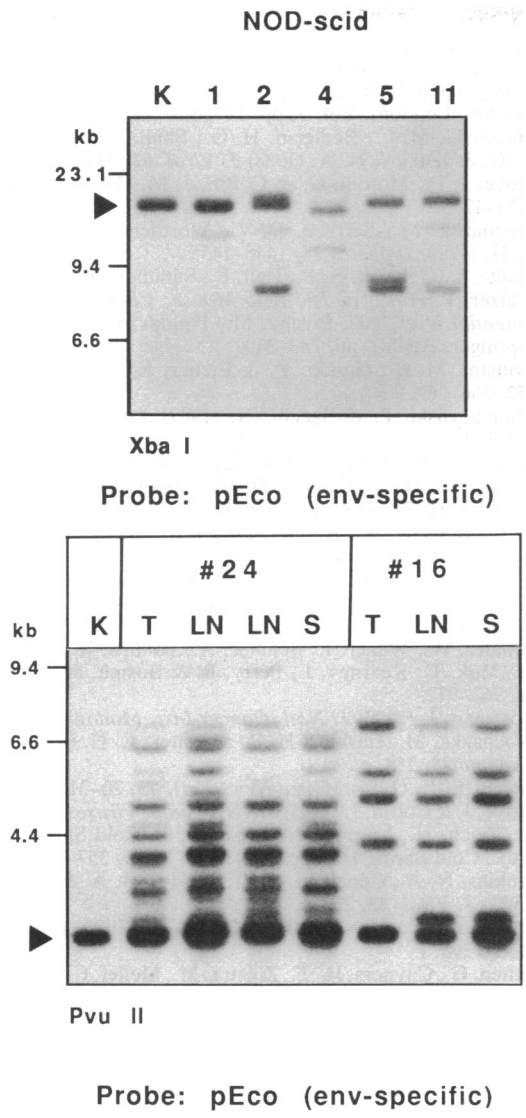

FIG. 3. Southern blot analysis of ecotropic proviral integrations in NOD-*scid*/*scid* tumors. Blots of DNA digested with *Xba* I (*Upper*) or *Pvu* II (*Lower*) were prepared as described in text and hybridized with ^{32}P-labeled pEco-*env* probe. (*Upper*) Comparison of NOD-*scid*/*scid* kidney (lane K) DNA with thymic lymphomas from five different individuals (lanes 1, 2, 4, 5, and 11). (*Lower*) Comparison of proviral integration patterns in multiple lymphoid tumors [thymic lymphoma (lanes T), lymph node tumor (lanes LN), spleen tumor (lanes S)] in two NOD-*scid*/*scid* mice (nos. 24 and 16). DNA samples from two LN tumors were analyzed for mouse no. 24. Lane K contains NOD-*scid*/*scid* kidney. The endogenous *Emv-30* fragment is marked by an arrowhead. Sizes of phage λ *Hin*dIII DNA markers are indicated on the left margin.

new proviruses detected in lymph node and spleen tumor DNAs, but not in thymic lymphoma DNA, of mouse 16 or 24 (Fig. 3 *Lower*) were apparently acquired at a later growth stage of the primary tumor or during the metastatic process. Alternatively, these neoplasms may have been polyclonal in origin.

MuLVs contribute to oncogenic transformation by integrating at or near cellular protooncogenes involved in the control of cell proliferation and differentiation, resulting in oncogene activation. The most frequently rearranged protooncogenes caused by proviral integrations in T-cell lymphomas are *Pim-1* and *Myc* (27, 28). No rearrangements at *Myc* loci were detected in Southern blots of DNA from 18 thymic lymphomas hybridized with a *Myc* probe. Using restriction enzymes (*Eco*RI, *Xba* I) suitable for detection of proviral insertions in the *Pim-1* locus on chromosome 17, we found rearrangements in 7 of 18 NOD-*scid*/*scid* tumors (data not shown). These rearrangements were not MCF-dependent because MCF integrations were detected in only 3 of the 7 tumors exhibiting *Pim-1* rearrangements, while *Emv-30* integrations were present in all 7 of them. In 2 of the tumors exhibiting *Pim-1* rearrangements, identical fragments corresponding to the altered *Pim-1* fragments were also detected with the pEco probe (not shown), indicating a direct association of *Pim-1* rearrangements with the proviral integrations.

DISCUSSION

Several features associated with both the NOD inbred strain genetic background and the defect imparted by the *scid* mutation make the NOD-*scid* mouse a unique model of thymomagenesis. The *scid* mutation engenders immunodeficiency by preventing normal rearrangement of functional TCR and immunoglobulin genes by an as yet undetermined mechanism (21). This mutation disrupts gene rearrangement in such a way that aberrant deletions occur in DNA regions where TCR gene assembly occurs. Thus, it is likely that a defective or missing recombinase system results from the *scid* mutation (29). That the recombinase system underlying immunoglobulin and TCR gene rearrangements is also utilized in DNA repair processes is indicated by demonstration of increased susceptibility to ionizing radiation-induced DNA damage in *scid*/*scid* mice (30–33). Since the *scid* mutation is associated with a defect in DNA repair (30, 32), the inability of *scid*/*scid* homozygotes to correct DNA breaks represents a high risk for tumor development and also may enhance oncogenic integrations of proviral DNA. However, genes at loci other than *scid* may also contribute to timing and frequency of *scid*-induced tumorigenesis. Indeed, the present results demonstrated a marked strain-dependent difference in thymic lymphoma incidence between the 67% incidence in NOD-*scid*/*scid* mice versus a reported 15–20% in the original C.B-17 background on which the mutation occurred (8). Differential endogenous proviral genes often account for strain-specific susceptibility or resistance to spontaneous lymphomagenesis.

A high incidence of spontaneous thymic lymphoma development in certain strains of mice, most notably AKR/J, has been explained in part by inheritance of certain endogenous ecotropic proviruses (34). However, in AKR/J thymic lymphomas, the ecotropic provirus is not the proximal agent of tumor induction, but rather, viruses formed by recombination between an ecotropic virus and one or more endogenous nonecotropic proviral sequences appear to be the causative agents (12, 35–37). In the present study, neither MCF nor ecotropic viral integrations into genes previously associated with lymphomagenesis in mice (*Pim-1*, *Myc*) were consistently observed. Furthermore, no single integrated ecotropic fragment was consistently associated with tumorigenesis. However, the presence of one or more acquired ecotropic fragments in all tumors (Fig. 3; also data not shown) was consistent with selective clonal expansion of neoplastic cells because of viral integration. A number of additional common ecotropic virus integration sites have been identified (38, 39) that were not explored in this study; certain of these could represent new common integration sites accounting for a subset of the observed thymic lymphomas. Other mechanisms of tumor initiation, in addition to insertional activation of protooncogenes, have been reported, including direct activation of growth factor receptors by viral proteins (40–43).

If interaction between the *scid* mutation and an expressible endogenous ecotropic provirus alone were required to initiate thymomagenesis, then a comparable incidence in NOD ver-

sus C.B-17 (a congenic stock of BALB/c) might be anticipated since the BALB/c genome also harbors a full-length ecotropic provirus [*Emv-1* on chromosome 5 (26)]. This provirus is present in C.B-17 (M.P., unpublished data) and is potentially expressible. Since the thymic lymphoma incidence is much lower in C.B-17-*scid/scid* mice, presumably other factors unique to the NOD inbred strain background facilitate the high spontaneous thymic lymphoma incidence. NOD mice are Fv-1^n type (H. G. Bedigian, personal communication), whereas C.B-17 mice are Fv-1^b type. Thus, NOD cells would be permissive to infection by an N-tropic ecotropic virus, whereas C.B-17 would not. Strain-dependent differences in the thymic microenvironment may also be contributory to the higher thymic lymphoma incidence on the NOD genetic background. Defects in NOD thymus structure and in the function of cortical thymocytes have been reported (7, 44, 45). In addition, NOD/Lt mice exhibit an unusual accumulation of T cells in peripheral lymphoid organs (6). Based upon the phenotype of the thymic lymphoma cells in NOD-*scid/scid* mice (the majority being Thy-1^+, $CD4^+$, $CD8^+$ double positive, $CD3^-$, IL-$2R^-$), it seems likely that, in these mutants, T-cell differentiation is blocked at an early stage of maturation in which immature T cells are in a stage of rapid proliferation (43). Since replication capacity decreases as cells differentiate, decreased differentiation potential elicited by the *scid* mutation should provide a large population of pre-T-cells with increased replication capacity (46). The differences in *Emv-30* expression observed between NOD-*scid/scid* and standard NOD/Lt mice may indeed reflect differences in differentiation stage-dependent gene expression. NOD/Lt is a highly susceptible strain for development of lymphomas and a variety of other neoplasias, but thymic lymphoma development has not been observed in standard NOD/Lt mice (10). Perhaps thymic defects in NOD mice synergize with the block in thymocyte differentiation imparted by the *scid* mutation to effect the high incidence of thymomagenesis on the NOD background. Exploration of the genetic basis underlying thymomagenesis in NOD-*scid/scid* mice may elucidate the basis for T-cell excess in NOD/Lt and demonstrate the importance of this phenotype for susceptibility to diabetes.

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