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Genetics

DNA sequence associated with chromosome translocations in mouse plasmacytomas

[non-immunoglobulin-associated rearranging DNA (NIARD)/chromosome translocations *T*(12;15) and rcp*T*(12;15)/mouse–hamster somatic cell hybrid/Southern transfer]

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ABSTRACT **A DNA sequence that generates aberrantly rearranged immunoglobulin heavy chain constant region genes in murine plasmacytomas is shown to participate in a chromosome translocation. We have previously termed this DNA sequence NIARD for non-immunoglobulin-associated rearranging DNA. NIARD rearrangements were found frequently in murine plasmacytomas but were not detected in normal lymphocytes. These rearrangements occasionally involve the switch region of the C_α gene. In this study, DNA samples obtained from mouse–Chinese hamster somatic cell hybrid lines were digested with various restriction endonucleases and analyzed by the Southern transfer technique with a NIARD hybridization probe. These experiments show that NIARD resides on chromosome 15 in the mouse germ line. Since NIARD is found adjacent to the C_α gene (located on chromosome 12) in some plasmacytomas, it is apparent that a translocation involving these two chromosomes has occurred. We have proposed a rcp*T*(12;15) model to explain our data. The implications of NIARD rearrangements for malignant transformation are discussed.**

Immunoglobulin gene loci display a unique potential for genetic recombination. The somatic recombination of Ig variable and constant region genes in B lymphocytes is a common feature of the three Ig gene families [λ and κ light chains and heavy chains (1–5)]. A second class of Ig gene rearrangements is responsible for a switch in expression from μ to one of six other heavy chain constant region (C_H) genes (i.e., γ_3, γ_1, γ_{2b}, γ_{2a}, ε, and α) during B-lymphocyte differentation (5–7). Tandemly repeated DNA sequences located ≈2 kilobases (kb) 5′ of each C_H gene, termed S regions, have been implicated in the C_H class switch (6–10). In mouse plasmacytomas, switch–recombination events result in the deletion of C_H genes 5′ of the expressed C_H gene (11–14).

Aberrant or nonfunctional C_H gene rearrangements likewise involve S regions and are commonly observed in mouse plasmacytomas (15–17). Some abortive C_H gene rearrangements can be simply explained by nonproductive inter-S region recombination (8, 16). We have recently shown that a novel class of aberrantly rearranged C_α genes is generated by a DNA recombination event between the S_α region and a DNA sequence not associated with any C_H gene (15). We have cloned this DNA sequence and refer to it as NIARD for non-immunoglobulin-associated rearranging DNA (see Fig. 1; ref. 15). NIARD rearrangements were observed in 15 out of 20 plasmacytomas tested including γ_3, γ_1, γ_{2b}, γ_{2a}, and α producers. In 2 out of these 15, NIARD was juxtaposed with the S_α region, while in the remainder NIARD rearranged with DNA sequences outside of the C_H gene locus. NIARD-mediated DNA rearrangements were not observed in normal lymphocytes.

In the study reported here, we have used mouse–Chinese hamster somatic cell hybrid lines to ascertain the chromosomal origin of the NIARD sequence. These hybrids have been successfully used for mapping a number of mouse genes to specific chromosomes (for review, see ref. 18).

MATERIALS AND METHODS

Somatic Cell Hybrids. Mouse–Chinese hamster somatic cell hybrid lines ECm4e [L cell–E36 Chinese hamster fibroblast (19)], MAE28 [Meth A cell–E36 (20)], and MACH 2A2 [A/HeJ mouse macrophage–E36 (21)] were generated, propagated in monolayer culture, and characterized as described (22). The three sublines MACH 2A2-B1, -C2, and -H3 were generated by plating MACH 2A2 cells at limiting dilution and picking individual colonies using cloning rings. Karyotypic and isoenzyme analyses to determine the mouse chromosome complement of a hybrid cell line were carried out on samples from the same cell population used for DNA preparation (22).

Southern Transfer and Hybridizations. DNA was digested with restriction endonucleases, electrophoresed through 1.2% agarose gels, and transferred to nitrocellulose filters essentially as described (16). The filters (see Fig. 2) were prehybridized for 6 hr at 42°C in 7.5 ml of 0.75 NaCl/0.075 M Na citrate/25 mM Na phosphate, pH 6.5/0.02% bovine serum albumin/0.02% Ficoll 400/0.02% polyvinylpyrrolidone/50% formamide/1% glycine containing sheared salmon sperm DNA at 250 μg/ml (23). Hybridization was for 2 days at 42°C in 5 ml of the above buffer without glycine and supplemented with 10% dextran sulfate and nick-translated probe at 2×10^6 cpm/ml (23). Filters were then washed for 15 min at room temperature in three changes of 0.30 M NaCl/0.03 M Na citrate/0.1% $NaDodSO_4$ and for 30 min at 50°C in two changes of 15 mM NaCl/1.5 mM Na citrate/0.1% $NaDodSO_4$ (23). Filters were exposed to Kodak XAR-5 film with an intensifying screen.

RESULTS

We have previously shown that our NIARD probe hybridizes to BALB/c liver DNA digested with restriction endonucleases *Eco*RI, *Hin*dIII, and *Bam*HI giving characteristic single bands of 22 kb, 5.1 kb, and 5.6 kb, respectively (15). This result, together with our previous analyses of a variety of plasmacytomas (PC3386, PC2149, PC3609, PC3612, and PC8701) suggests that NIARD is a unique sequence in the mouse genome (15). To determine the germ-line chromosomal location of the NIARD sequence, we scored somatic cell hybrids containing various

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Abbreviations: NIARD, non-immunoglobulin-associated rearranging DNA; C_H and V_H, Ig heavy chain constant and variable region genes, respectively; kb, kilobase(s).

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Table 1. Chromosome content and NIARD reactivity of mouse–Chinese hamster somatic cell hybrids

Mouse chromosome	MAE28	ECm4e	MACH 2A2-B1	MACH 2A2-C2	MACH 2A2-H3
1	0.00	0.00	0.80	0.50	0.65
2	0.00	0.00	1.10	0.60	0.00
3	0.00	0.00	0.75	0.45	0.75
4	0.00	0.00	0.35	0.10	0.15
5	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.60	0.00	0.80
7	0.00	0.00	0.45	0.55	0.85
8	0.00	0.00	0.60	0.40	0.90
9	0.00	0.00	0.80	0.85	0.90
10	0.00	0.00	0.70	0.50	0.75
11	0.00	0.00	0.00	0.00	0.00
12	1.00	0.00	1.00	0.90	1.40
13	0.00	0.00	0.00	0.45	0.75
14	0.00	1.00	1.30	0.00	1.15
15	0.00	1.00	0.65	0.35	1.30
16	0.00	0.00	0.45	0.65	0.55
17	0.00	0.00	0.50	0.85	1.05
18	0.00	0.00	0.00	0.00	0.60
19	0.00	0.00	0.00†	0.50	0.95
X	1.00	0.00	0.35	0.60	0.70
Cells karyotyped, no.	50	20	20	20	20
NIARD response	−	+	+	+	+

The number of copies of each mouse chromosome per diploid Chinese–hamster complement is given, and the hybridization response with a NIARD probe is indicated at the bottom of each column.

combinations of mouse chromosomes on a constant Chinese hamster background for the presence or absence of NIARD. The mouse chromosomal contents of the somatic hybrid cell lines are shown in Table 1. DNA samples isolated from hybrid cell lines were digested with *Eco*RI, *Hin*dIII, and *Bam*HI and hybridized by Southern transfer to a NIARD DNA probe (Fig. 1). The hybridization results are shown in Fig. 2 and summarized in Table 1. No NIARD-reactive sequences were found in E36 (Chinese hamster) DNA. Hybrid MAE28, which contains mouse chromosomes 12 and X, yielded no NIARD sequence. Hybrid ECm4e, however, yielded NIARD sequences, as did hybrids MACH 2A2-B1, -C2, and -H3. The only mouse chromosome present in all of the NIARD-positive hybrids was number 15.

DISCUSSION

Because NIARD resides on chromosome 15 while the heavy chain genes are located on chromosome 12 in the mouse germ line (24, 25), the juxtaposition of NIARD and the C_α gene in certain plasmacytomas [e.g., J558 and TEPC 601 (15), M603 and M167 (26), S107 (17)] implies that a translocation involving chromosomes 12 and 15 has occurred. In reviewing the cytogenetics of chromosome translocations in mouse plasmacytomas, we found some remarkable similarities to NIARD rearrangements that lead us to postulate that NIARD may be involved in the translocations *T*(**12;15**) and rcp*T*(**6;15**), which are so frequently seen: (*i*) the germ-line origin of NIARD is on chromosome 15; (*ii*) at least one germ-line copy of NIARD remains in all plasmacytomas (15) and one copy of chromosome 15 does not undergo translocation (27–31); (*iii*) NIARD rearrangements are not seen in hybridomas (15) and chromosome 15 translocations are not found in normal spleen cells; (*iv*) NIARD sometimes rearranges with the S_α region (15, 17, 26) and the distal end of chromosome 15 sometimes translocates to the band of chromosome 12 containing the Ig heavy chain genes (12F1) (25, 27–31); and (*v*) all λ producers tested have rearrangements with S_α (15, 17, 26), and in λ producers, translocations are always of the *T*(**12;15**) type and never of the rcp*T*(**6;15**) type (28). In light of these arguments, we have drawn a model (Fig. 3A) showing the putative involvement of NIARD rearrangements in the *T*(**12;15**) (F2; D3/E) described by Ohno *et al.* (27). In the germ line, we have placed NIARD at the breakpoint on chromosome 15 (band D3/E) and the C_α gene at the breakpoint (terminus) of chromosome 12. When the translocation occurs, NIARD and

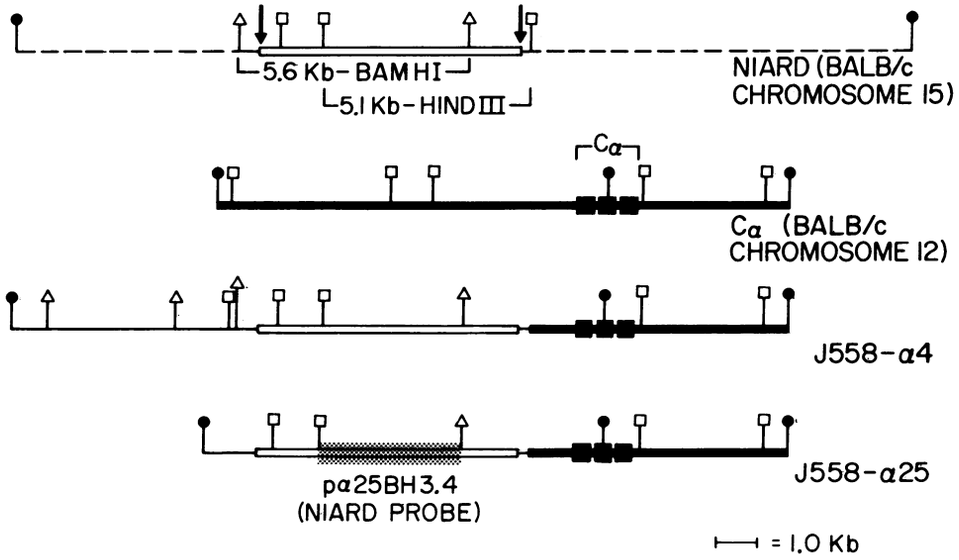

FIG. 1. NIARD generates aberrantly rearranged α genes by chromosomal translocation. Endonuclease sites: ●, *Eco*RI; △, *Bam*HI; □, *Hin*dIII. Open rectangles indicate the DNA segment from germ-line NIARD that rearranges into both J558-α4 and J558-α25 (two abortively rearranged α genes). Filled rectangles indicate sequences found in germ-line C_α. The broken line indicates germ-line NIARD fragment lengths inferred from the 5′ *Eco*RI site of J558-α4. Filled squares indicate the three coding regions of C_α. The stippled rectangle indicates the 3.4-kb *Bam*HI/*Hin*dIII fragment used as a NIARD probe in Southern transfer hybridization experiments. Heavy arrows indicate rearrangement sites within NIARD.

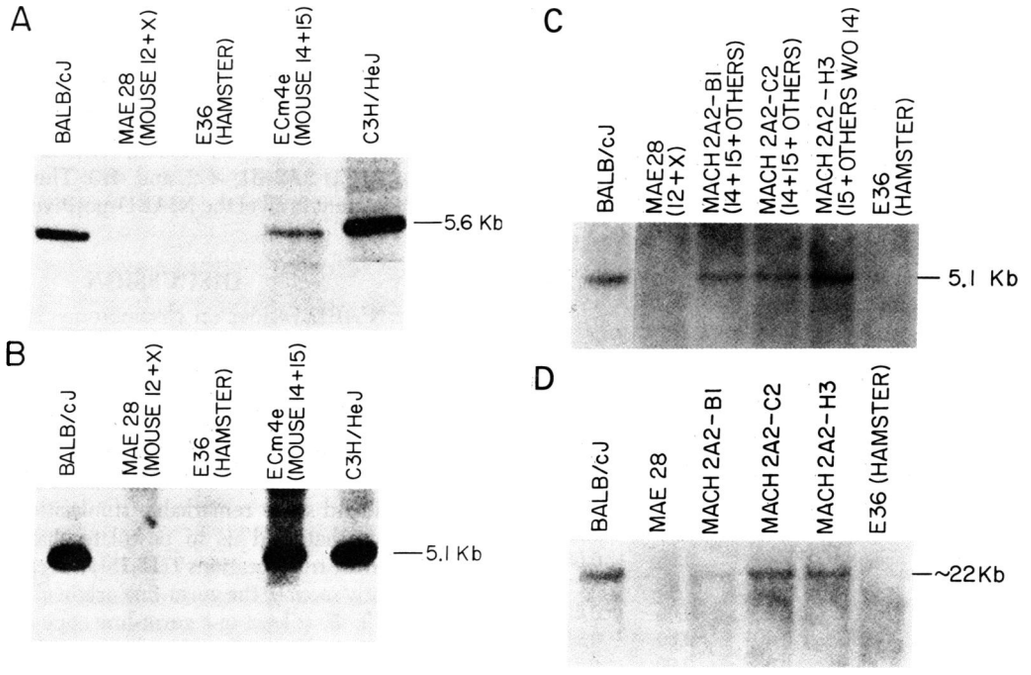

FIG. 2. Southern transfers of *Bam*HI (*A*), *Hin*dIII (*B* and *C*), and *Eco*RI digestions of mouse–Chinese hamster somatic cell hybrid DNAs hybridized to the NIARD probe (pα25BH3.4).

C_α become juxtaposed. This model has two problems. First, Meo *et al.* (25) have localized the Ig heavy chain genes at or proximal to band 12F1 (i.e., the C_H genes are located at band 12F1 or between this band and the centromere), yet Ohno *et al.* (27) place the breakpoint at 12F2. Second, in this model the constant region genes are proximal to the variable region genes. This positioning is inferred from the apparent translocation of the distal portion of chromosome 15 (presumably containing NIARD) to the terminus of chromosome 12. However, at the molecular level, we see that NIARD translocated to a position 5′ of C_α. This directly contradicts the placement of the heavy chain variable region (V_H) genes both proximal to the C_H genes on the Mendelian map (32–34) and 5′ of the C_H gene cluster at the DNA level.

In Fig. 3*B*, we present an alternative model depicting the putative involvement of NIARD and C_α in a rcp*T*(**12;15**). The juxtaposition of NIARD and C_α occurs by translocation of the distal part of chromosome 12 (containing C_α at the breakpoint) to the distal part of chromosome 15 (containing NIARD at the breakpoint). We have moved the breakpoint on chromosome 12 to band 12F1 and reversed the orientation of the Ig-h genes so that the V_H genes are proximal to the C_H genes. Therefore, this model does not dispute the results of either Meo *et al.* (25), Riblet (32), or Owen *et al.* (33, 34). In fact, although Ohno and co-workers have described a *T*(**12;15**) translocation, they stated that the placement of the breakpoint at 12F2 was "not unequivocal" and that the *T*(**12;15**) marker might have been due to a reciprocal translocation between chromosomes 12 and 15. We have yet to demonstrate the existence of a NIARD-associated DNA sequence adjacent to any remaining C_H gene on chromosome 12. Moreover, in the case of J558, the μ and γ genes are deleted from the genome while multiple rearranged heavy chain joining region sequences are present (unpublished results). Further evidence that this is the translocation seen by cytogenetics would be provided by a direct correlation of the cytogenetic and Southern hybridization data [i.e., to verify that NIARD rearranges with S_α in plasmacytomas displaying a *T*(**12;15**) but does not rearrange in the absence of a translocation]. Because chromosome 6 contains the κ light chain genes (35), the rcp*T*(**6;15**) translocation may be a rearrangement of a NIARD-like sequence to the C_κ locus. This latter translocation may occur in all or some of the plasmacytomas in which NIARD rearranges outside of the C_H locus (15). Alternatively, the site of the non-C_α gene-associated NIARD rearrangements may reside elsewhere on chromosome 12.

We have recently observed a NIARD rearrangement in a monocytic leukemia, suggesting that chromosomal aberrations generated by NIARD may be associated with tumors other than plasmacytomas (unpublished results). Since NIARD rearrangements are found in more than one type of tumor, it seems possible that NIARD rearrangements may play a causative role in malignancy. The mechanism(s) by which this may occur remains unknown, but some possibilities are (*i*) intact NIARD has a suppressive effect on malignancy and the loss of an intact copy of NIARD results in expression of the malignant phenotype; (*ii*) intact NIARD is normally inactive, but its translocation results in the formation of an expressible gene that promotes malignancy; and (*iii*) the translocation of NIARD results in the inactivation of a gene that normally suppresses the malignant phenotype.

Nonrandom chromosome translocations have been associated with proliferative diseases of the hematopoetic system in humans and animals. In man, the Philadelphia chromosome (Ph^1) (a translocation of the distal part of 22q to 9q) is associated with chronic myelogenous leukemia (36, 37). A *T*(**8;14**)(q23;q32) is associated with Burkitt lymphoma (38, 39) and B-cell acute lymphocytic leukemia (40). The human Ig heavy chain gene locus has recently been localized by direct *in situ* hybridization to chromosome 14 (band q32) and may therefore participate in *T*(**8;14**) (41). In mice, trisomy of the distal part of chromosome 15 is present in a majority of T-cell leukemias or lymphomas, including those occurring spontaneously in AKR mice (42) and those induced by chemicals, x-rays, and viruses (43–47).

To our knowledge, NIARD represents the first example of a specific segment of DNA that has been localized to the site of a chromosome translocation in a mammalian cell. Further

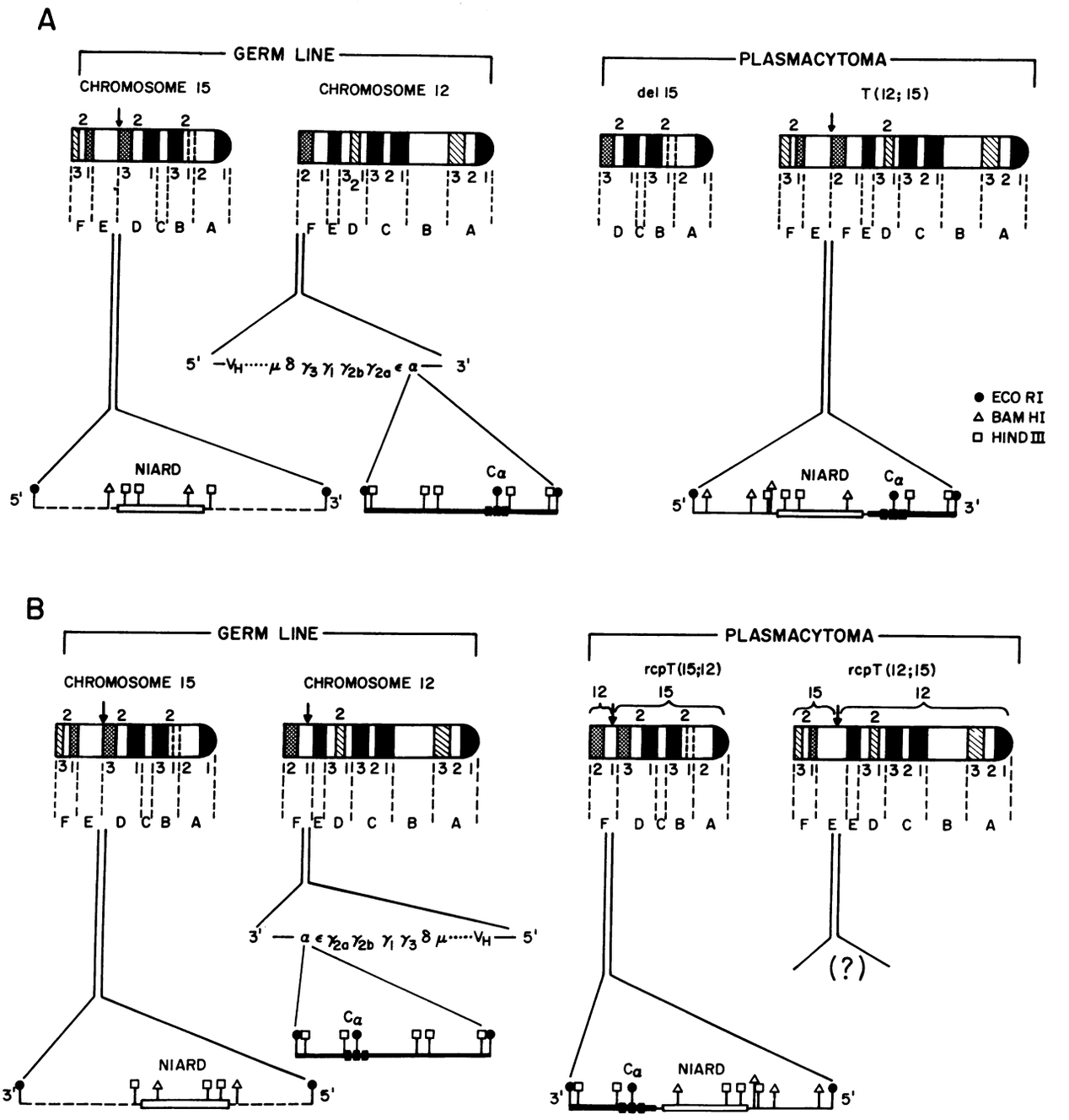

FIG. 3. Diagrams of models of NIARD rearrangements. (*A*) Simple *T*(**12;15**) translocation: the DNA distal to the breakpoint 15D3/E translocates to the distal end of chromosome 12. NIARD is placed at 15D3/E and the Ig-h genes are at 12F2 so that our molecular data will fit the schematic representation suggested by Ohno *et al.* (27). Note that the Ig-h genes are oriented so that the V_H genes are distal to the C_H genes. (*B*) rcp*T*(**12;15**) translocation: the DNA distal to the breakpoint at 15D3/E translocates to 12F1 and the DNA distal to 12F1 translocates to 15D3/E. NIARD is placed at 15D3/E in order for the molecular data to fit this schematic drawing. The Ig-h genes are placed at 12F1, according to Meo *et al.* (25), and the Ig-h genes are oriented so that the V_H genes are proximal to the C_H genes. In plasmacytomas, no data exist for the arrangement of the remaining C_H genes on chromosome 12 or for the existence of NIARD-associated DNA in the segment translocated from 15 to 12.

experiments with NIARD should give us some insight into the role of chromosome translocations in malignant diseases.

Note Added in Proof. While this manuscript was in press, we were informed by K. Calame of confirmatory results to those reported here that were obtained with a DNA probe analogous to NIARD isolated from a MOPC 603 aberrant C_α gene (see ref. 26).

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