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Immunology

Non-immunoglobulin-associated DNA rearrangements in mouse plasmacytomas

[non-immunoglobulin-associated rearranging DNA (NIARD)/C_α gene rearrangements/heavy chain switch sequences/chromosome translocations]

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ABSTRACT **We have characterized a class of DNA rearrangements in plasmacytomas. These recombination events involve a DNA sequence whose origin is outside of the locus of the heavy chain constant region genes, C_H. Therefore, we choose to refer to this sequence as non-immunoglobulin-associated rearranging DNA (NIARD). We have isolated two abortively rearranged C_α genes, generated by NIARD events from the α-producing J558 myeloma. Restriction endonuclease maps of these sequences reveal two possible recombination sites in NIARD that are separated by ≈6.5 kilobase pairs of DNA (defined as 5′ and 3′ sites). A NIARD rearrangement occurs in 15 out of 20 plasmacytomas tested, including γ3-, γ1-, γ2b-, γ2a-, and α-producers, but this event usually does not involve a C_H switch (*S*) region. In fact, only S_α appears to accept NIARD. However, NIARD did not undergo a rearrangement in eight IgA-producing hybridomas tested. One germ-line copy of NIARD (a 22-kilobase pair *Eco*RI fragment) is retained in all plasmacytomas. NIARD does not appear to possess repetitive DNA sequences homologous to S_μ or S_α. We discuss the possible role and implications of NIARD-like sequence rearrangements in allelic exclusion and chromosomal translocation events in plasmacytomas.**

Switches in the expression of immunoglobulin heavy chain constant region (C_H) genes occur via a unique class of DNA recombination events in immature B lymphocytes (1–5). DNA deletion events have been associated with the C_H class switch mechanism in the mouse myeloma system (6–8). These C_H gene deletion events have allowed a number of investigators to construct a map of the C_H gene locus (6, 7), which has been substantiated by direct molecular linkage analyses (9, 10). The C_μ gene is the most 5′ C_H gene (6, 9, 10–12) and is expressed first in pre-B lymphocytes (1, 2). Switches from C_μ to any of six other C_H genes (i.e., γ3, γ1, γ2b, γ2a, ε, or α) appear to occur only via DNA rearrangements that result in the deletion of the C_H genes 5′ of the expressed C_H isotype (6–8). These rearrangement events have been proposed either to occur by intrachromosomal deletions (6) or to be the result of sister chromatid exchanges (13). A class of short tandemly repeated DNA sequences localized about 1.5–2.0 kilobase pairs (kbp) 5′ of each C_H gene has been implicated in the switching mechanism (4, 14–18) and termed switch (*S*) regions (4).

Several models have been proposed for the regulation of the C_H gene switch (7, 14, 16, 18). One of these ideas suggests that the C_H class switch is probably a stochastic or random process (7). Functional as well as nonfunctional events would be predicted in such a phenomenon (8, 17). In fact, abortive C_H switch events have recently been characterized for a γ2b gene in MPC-11 cells (15, 17) and an α gene in S107 (19). In the case of the aberrant MPC-11 γ2b gene, this event was caused by an inter S region recombination between $S_{\gamma 3}$ and $S_{\gamma 2b}$ that resulted in the deletion of the γ3 and γ1 genes (15, 17). In S107, a DNA sequence of unknown origin recombined with the C_α switch region (19).

In this report, we describe a class of DNA rearrangement events occurring in mouse plasmacytomas that are mediated by a non-C_H gene-associated DNA sequence. We refer to this DNA sequence as NIARD for non-immunoglobulin-associated rearranging DNA. NIARD originates from outside of the C_H gene locus. This sequence undergoes a variety of rearrangement events that occasionally involve the S_α region but do not appear to require the direct participation of a C_H *S* region.

MATERIALS AND METHODS

Southern blot hybridizations (20) and molecular cloning were performed as described (12, 21).

RESULTS

Two Abortively Rearranged Genes in the J558 Myeloma. Restriction maps of two abortively rearranged α genes isolated from a Charon 4A λ phage (22) partial *Eco*RI library of J558 myeloma DNA (J558 α4 and α25) are compared to the BALB/c germ-line α gene in Fig. 1. A DNA sequence of unknown origin has recombined with the S_α region in α4 and α25, presumably inactivating the C_α gene for any further rearrangement events. The functional C_α gene in J558 has also been isolated and found to possess V_H (variable), J_H (joining), and S_μ sequences and will be described in detail elsewhere.

Two Independent Recombination Events Generate the J558 α4 and α25 Clones. In order to compare the α4 and α25 DNA rearrangements, cross-hybridization experiments were performed between the 5′ end of α4 (pα4RB0.9), the S_α recombination site of α4 (pα4RB3.5), the sequences 5′ of the S_α rearrangement site in α25 (pα25BH3.4), and a subclone-containing germ-line S_α and Cα(pα64-101) as shown in Fig. 2. These and other hybridization experiments indicate that: (*i*) A portion of the 3′ end of the S_α sequence is retained in the α4 and α25 recombination regions. The comparable, strong hybridization response observed for rearranged and germ-line α genes with a combined $S_\alpha + C_\alpha$ probe (p64-101) indicates that repetitive S_α sequences are retained in α4 and α25 (see Fig. 2*A*). In addition, we have also found at least 300 bp of S_α 5′ of the *Xba* I site in pα4RB3.5 (unpublished results). (*ii*) Approximately 6.5 kbp 5′

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Abbreviations: C_H, V_H, and J_H, genes for constant, variable, and joining regions of immunoglobulin heavy chains; *S* region, switch region; kbp, kilobase pair(s); NIARD, non-immunoglobulin-associated rearranging DNA.

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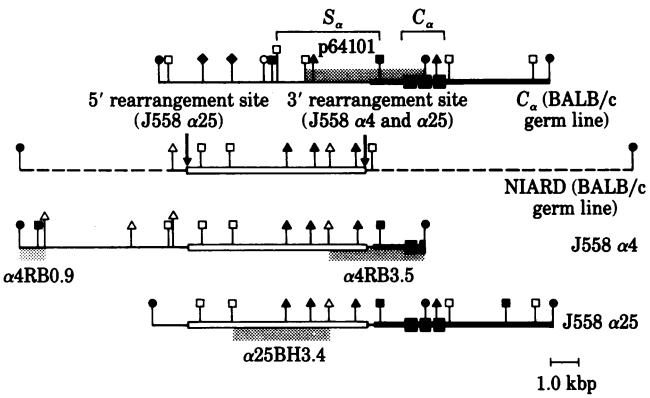

FIG. 1. NIARD generates two abortively rearranged C_α genes in the J558 myeloma. Endonuclease sites are ●, *Eco*RI; ▲, *Sac* I; △, *Bam*HI; □, *Hin*dIII; ■, *Xba* I; ◆, *Bgl* II; ○, *Kpn* I. Filled rectangles indicate C_α germ-line sequences, open rectangles indicate NIARD common sequences, and the broken line indicates germ-line NIARD fragment lengths inferred from the 5′ *Eco*RI site of J558 α4.

of the S_α recombination sites in α4 and α25 are identical. (*iii*) The DNA sequences 5′ of this 6.5-kbp common region display no homology to α4 recombination fragment (i.e., pα4RB3.5) because α4RB3.5 detectably hybridizes only to itself. (*iv*) The 5′ end of α4 does not hybridize to the 5′ end of α25. These findings can be used collectively to argue that a minimum of two independent DNA recombination events generated α4 and α25 and that the 5′ and 3′ recombination sites display no significant

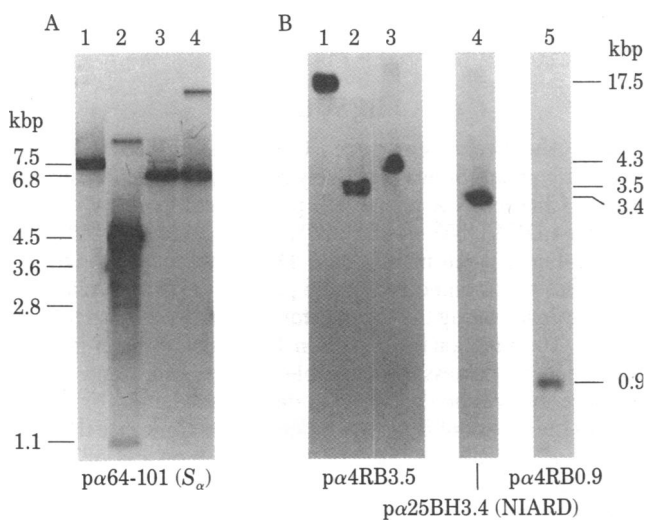

FIG. 2. Southern transfers of genomic clones and genomic DNAs hybridized to pα64-101, pα4RB3.5, and pα4RB0.9 probes. (*A*) Track 1, *Hin*dIII-digested α25 DNA; track 2, *Eco*RI/*Hin*dIII-digested M64 (BALB/c germ-line S_α- and C_α-containing genomic clone) (12); tracks 3 and 4, *Eco*RI/*Hin*dIII digested α25 and α4, respectively. (*B*) Track 1: *Bam*HI/*Hin*dIII-digested α4; track 2, *Bam*HI/*Eco*RI-digested α25; track 3, *Bam*HI/*Hin*dIII-digested α25; track 4, *Bam*HI/*Hin*dIII-digested α4; and track 5, *Eco*RI/*Bam*HI-digested α4. The two intensely hybridizing bands in track 3 of *A* are S_α sequences, and other minor bands in the M64α sample are due to the genetic instability of the S_α sequences (12, 21). The discrepancy in the sizes of the *Bam*HI/*Hin*dIII fragments that hybridize to pα4RB3.5 probe in lanes 1 and 3 of *B* are due to the absence of a 3′ 4.5-kbp germ-line *Eco*RI fragment from the α4 clone. The 17.5-kbp fragment in lane 1 of *B* also contains a large portion of the Charon 4A left arm. The pα4RB0.9 probe in *B* was also hybridized to Southern transfers of restriction endonuclease-digested α25 clone but no detectable hybridization was observed in the α25 lane (data not shown).

sequence homology. α25 was generated from α4 not by a simple deletion event occurring at the 5′ end of α4 but by a second rearrangement event with a DNA sequence of unknown origin. A comparison of the 6.5-kbp common region 5′ of S_α shared by α4 and α25 (see Fig. 1) with the published restriction maps of all DNA sequences in the environment of other C_H genes, including their *S* regions and spacer sequences (9, 10), indicates that the germ-line origin of this sequence is not within the C_H gene cluster. Consequently, we choose to refer to the 6.5-kbp common region shared by α4 and α25 as NIARD and utilize pα25BH3.4 as a NIARD probe.

NIARD Rearrangements in Plasmacytomas. Southern transfers of restriction enzyme digestions (*Eco*RI, *Bam*HI, and *Hin*dIII) of individual examples of μ-, γ3-, γ1-, γ2b-, and γ2a-producing plasmacytomas were hybridized to the pα25BH3.4 NIARD probe and are displayed in Fig. 3 *A–C*. These results indicate that: (*i*) NIARD is found in both the BALB/c and the NZB germ lines as unique 22.0-, 5.6-, and 5.1-kbp bands upon *Eco*RI, *Bam*HI, and *Hin*dIII digestion, respectively (Fig. 3 *A–C* and Fig. 4*D*). (*ii*) Except for PC3741 (a μ producer), each of these myelomas contains at least one rearranged NIARD in addition to maintaining a germ-line NIARD sequence.

The most common NIARD rearrangement appears to occur within 1.5 kbp 3′ of the *Bam*HI site of pα25BH3.4 (see Fig. 1). This conclusion is based on the observation that most NIARD rearrangements are apparent upon *Eco*RI and *Hin*dIII digestion (Fig. 3 *A* and *C*) but not upon *Bam*HI digestion (Fig. 3*B*). *Bam*HI digestion yields a germ-line-sized NIARD band for this entire group of myelomas except for J558 that is due to the existence of J558 α25 (see Fig. 3*B*). Therefore J558 α25 would appear to have been created by at least two independent rear-

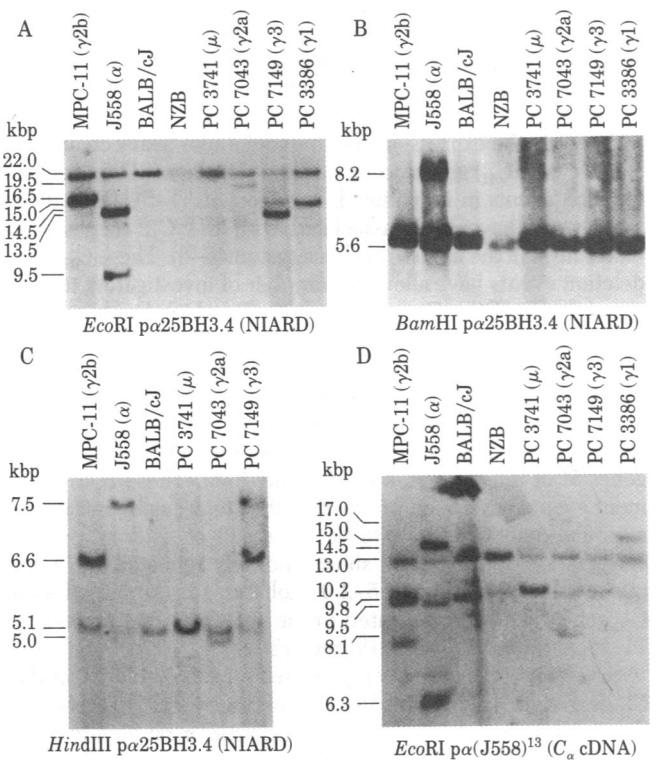

FIG. 3. Southern transfers of restriction enzyme digestions of myeloma and germ-line DNAs hybridized to NIARD and C_α probes. PC indicates a plasmacytoma. The 13.0-kbp band in the *D* lanes is due to a V_H gene that strongly hybridizes to the pα(J558)[13] probe because this reagent contains J558 V_H framework III sequences (12). The 9.8-kbp band in the BALB/c track of *D* corresponds to a C_α *Eco*RI fragment (12).

rangement events occurring immediately 5′ and 3′ of the ≈6.5-kbp α4 common region (see Fig. 1). These conclusions are completely in agreement with the genomic clone hybridization data presented in the prior section (Fig. 2). Because the α25 rearrangement and a remaining germ-line NIARD sequence would appear to be present in similar amounts in blots of J558 DNA, this germ-line copy of NIARD does not originate from contaminating lymphocytes. PC 7149 also contains evidence of two NIARD events, but both of these appear to be 3′ in nature.

These findings allow us to construct a restriction map of the germ-line NIARD sequence and also to position the approximate 5′ and 3′ rearrangement sites within germ-line NIARD (see Fig. 1). The locations of the 5′ and 3′ *Eco*RI sites of germ-line NIARD in Fig. 1 are tentatively placed, assuming that the context of the 5′ end of the α4 clone is germ line. NIARD rearrangements may occur beyond the boundaries indicated in Fig. 1 and not be apparent upon *Bam*HI and *Hin*dIII digestion. However, we have not observed a rearrangement upon *Eco*RI digestion in the absence of either a *Bam*HI or a *Hin*dIII rearrangement (see Fig. 3 and 4 and Table 1). Therefore, we can assume with some confidence that these rearrangements occur within 2.1 kbp 5′ and 1.5 kbp 3′ of α25BH3.4 as shown in Fig. 1.

NIARD Rearrangements Preferentially Occur Outside the C_H Gene Locus. We next attempted to ascertain whether either NIARD rearrangement was associated with a C_H S region. *Eco*RI-digested DNAs of this first group of myelomas were hybridized to an α cDNA clone, pα(J558)[13] (12). As expected, the 3′ portions of both NIARD events in J558 are associated with S_α. However, none of the remaining NIARD rearrangements appears to involve S_α.

The NIARD event in MPC-11 generates a 16.5-kbp *Eco*RI fragment, as shown in Fig. 3*A*, and this rearrangement does not involve the μ, γ3, γ1, γ2b, γ2a, ε, or α genes. From our previous work on MPC-11 DNA, we know that: (*i*) The S_μ region is completely deleted from the nonfunctionally rearranged MPC-11 chromosome (ref. 21; unpublished data) and the only remaining portion of S_μ is present in the functionally rearranged γ2b gene (17). (*ii*) The γ3 and γ1 genes are deleted from MPC-11 DNA (15, 17). (*iii*) In addition to a functionally rearranged γ2b gene, one abortively rearranged γ2b gene is also present due to an $S_{\gamma 3} \times S_{\gamma 2b}$ recombination event (15, 17). (*iv*) Two γ2a genes with rearranged $S_{\gamma 2a}$ sequences are found 3′ of these two γ2b genes, but their restriction fragment patterns are incompatible with the observed NIARD bands (17). (*v*) The size of the *Eco*RI fragment containing the two γ2a alleles in MPC-11 is in agreement with the size of the BALB/c germ-line γ2a *Eco*RI fragment (9, 10), indicating that the ε genes are 3′ of these γ2a genes in their germ-line state (9, 10, 23). In consideration of these observations, we decided to determine whether NIARD rearrangements preferentially occur without S region participation.

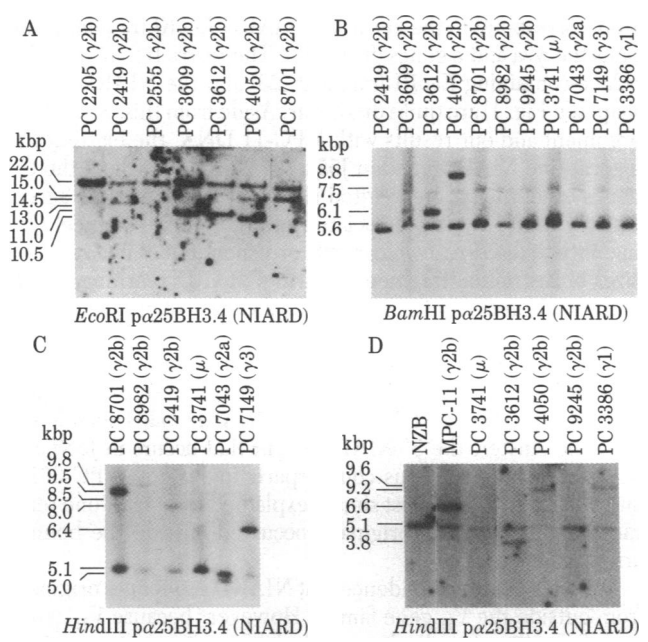

FIG. 4. Southern transfers of restriction enzyme digestions of various myeloma DNAs hybridized to NIARD.

NIARD events were searched for in an identical manner in 11 additional γ2b plasmacytomas, two α plasmacytomas, and eight α-producing hybridomas. A sample of the hybridization results is shown in Fig. 4 and a summary of all the NIARD data is presented in Table 1. Three γ2b producers and one α (MOPC 315) show no NIARD rearrangements. Five γ2b producers and one α (TEPC 601) show 3′ NIARD events. Three γ2b expressors show 5′ NIARD rearrangements. Two γ2b producers possess two independent 5′ and 3′ rearrangements (see PC 3612 and 4050 in Table 1). Similar experiments performed with eight IgA-producing hybridomas have revealed no additional NIARD events other than the stable rearrangement present in the fuser cell's genome (a selectable derivative of MPC-11 cells) (see Table 1). Hybridization experiments performed with our α cDNA probe indicate that only the TEPC 601 rearrangement involves S_α (data not shown).

Table 1. Compilation of all NIARD rearrangement data

DNA sample	State of NIARD sequences* Germ line	5′ events	3′ events	*Eco*RI only
PC 3741 (μ)	+	N	N	
8 IgA hybrids†	+	N	16.5R, 6.5H	
PC 7149 (γ3)	+	N	15.0R, 7.9H	
			13.5R, 6.6H	
PC 3386 (γ1)	+	N	15.0R, 9.6H	
MPC-11 (γ2b)	+	N	16.5R, 6.6H	
CAL9-PC1 (γ2b)	+	ND	ND	16.5R
PC 2205 (γ2b)	+	N	N	
PC 2419 (γ2b)	+	N	13.0R, 8.2H	
PC 2555 (γ2b)	+	N	N	
PC 3609 (γ2b)	+	11.0R, 6.1B	ND	
PC 3612 (γ2b)	+	11.0R, 6.1B	11.0R, 3.8H	
PC 4050 (γ2b)	+	10.5R, 8.8B	10.5R, 9.2H	
			14.5R, 6.6H	
PC 8701 (γ2b)	+	N	15.0R, 9.2H	
PC 8982 (γ2b)	+	N	15.5R, 9.8H	
PC 9245 (γ2b)	+	ND	ND	13.5R
PC 10916 (γ2b)	+	N	N	
PC 7043 (γ2a)	+	N	19.5R, 5.0H	
J558 (α)	+	9.5R, 8.2B	14.5R[α], 7.5H[α]	
			9.5R[α], 7.5H[α]	
TEPC 601 (α)	+	N	14.5R[α], 8.0H[α]	
MOPC 315 (α)	+	N	N	
SP2-0 (−)	+	N	ND	13.0R

* N, normal or no rearrangement; ND, not determined. R, *Eco*RI; B, *Bam*HI; and H, *Hin*dIII fragments detected, preceded by lengths given in kbp.

† Tumor samples of eight IgA-producing hybridomas (4521.1, 26.4.1, U10.D, X1B.1, 14.6B.1, X17B, 10.16.1, and 14.7.1). A drug-marked derivative of MPC-11 cells was used as the fuser cell line to prepare these hybridomas (24, 25). The single NIARD rearrangement detected in all of these hybridomas is contributed solely by the fuser cell.

To rule out the involvement of other C_H S regions in NIARD events in this large group of plasmacytomas, hybridization experiments were also performed with $S_{\gamma 3}$, $S_{\gamma 2b}$, and $C_{\gamma 2b}$ probes, which are capable of detecting γ3, γ1, γ2b, and γ2a genes and their S regions. Samples of some of these experiments are shown in Fig. 5. Comparisons of the sizes of *Eco*RI and *Bam*HI restriction fragments detected by $S_{\gamma 2b}$ and $S_{\gamma 3}$ probes in the NZB γ2b plasmacytomas with the corresponding DNA fragments observed with the NIARD probe (see Fig. 3 and 4 and Table 1) reveal no apparent similarities. These probes can reliably detect the $S_{\gamma 3}$, $S_{\gamma 2b}$, and $S_{\gamma 2a}$ regions in genomic Southern blots (ref. 17 and Fig. 5), but $S_{\gamma 1}$ is barely detectable (see low molecular weight *Bam*HI fragments in NZB tracks of Fig. 5*A*). However, the presence of germ-line $S_{\gamma 3}$ and γ3 sequences on the unexpressed chromosome in a number of these γ2b-producing plasmacytomas would suggest that the 5′ flanking region of the γ1 gene is intact and therefore not participating in a NIARD event.

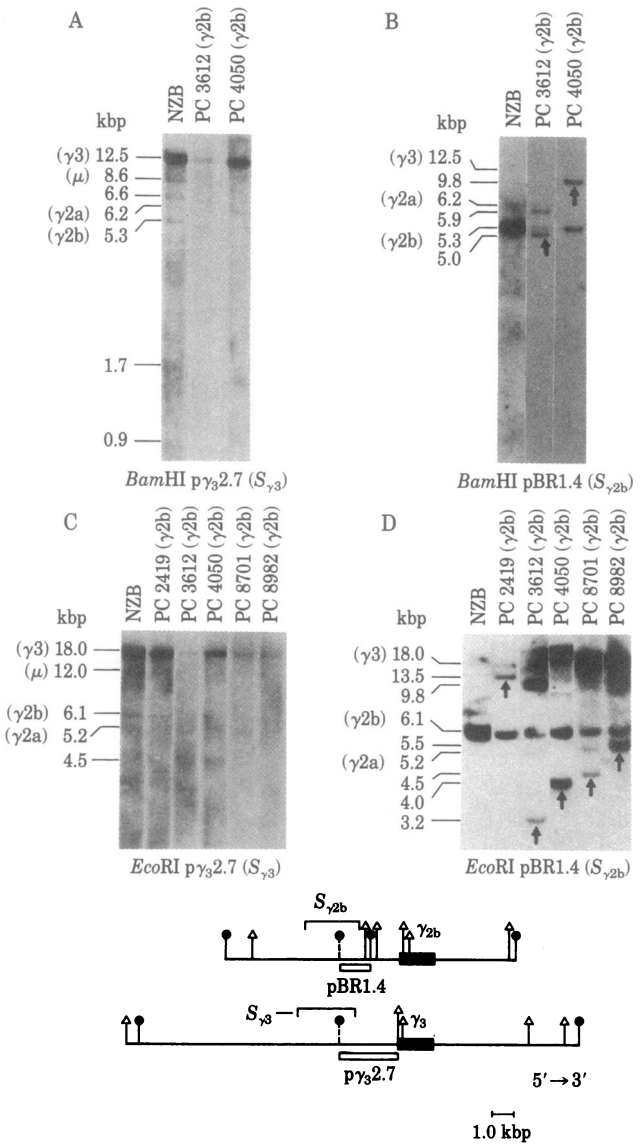

FIG. 5. Southern transfers of restriction enzyme digestions of DNAs from several NZB producing plasmacytomas hybridized to either $S_{\gamma 3}$ (p$\gamma_3$2.7) or $S_{\gamma 2b}$ (pBR1.4) probes. Detectable hybridization signals above 13.5 kbp in the PC 8701 and PC 8982 tracks of *D* are in part due to the 18-kbp germ-line γ3 gene (which is more clearly visualized in *C*) and an unusually high level of background noise. No bands corresponding in size to both *Eco*RI and *Bam*HI rearranged NIARD sequences (see Table 1) are observed with either $S_{\gamma 2b}$ or $S_{\gamma 3}$ probes in these representative plasmacytomas. Low molecular weight hybridizing bands (i.e., <2 kbp) in *A* correspond in part to the $S_{\gamma 1}$ and $S_{\gamma 2a}$ regions, which possess multiple *Bam*HI sites (9, 10). Arrows indicate the position of migration of the expressed γ_{2b} gene in these plasmacytomas. This was confirmed by J_H and S_μ probe hybridizations (unpublished results). Maps of the hybridization probes are shown at the bottom. ●, *Eco*RI sites; △, *Bam*HI site. The broken vertical line indicates a site artificially added in the cloning protocol.

DISCUSSION

We have described a class of DNA rearrangements occurring in mouse myelomas. We have termed the DNA sequence mediating this phenomenon NIARD, for non-immunoglobulin-associated rearranging DNA. NIARD events occasionally occur with the C_H S region, and this finding allowed us to prepare a NIARD probe from an abortively rearranged α gene clone (α25 in Fig. 1) isolated from the J558 myeloma. Rearranged α genes that are somewhat analogous to the α4 clone of J558 (Fig. 1) have recently been observed in the α-producing myelomas S107 (19) and MOPC 603 and M167 (K. Calame and S. Kim, personal communication). In the case of S107, the DNA sequence participating in the aberrant rearrangement of a C_α gene possesses a similar restriction map to our NIARD sequence (19). The S107 NIARD-like sequence would also appear to originate from outside the C_H gene cluster. The NIARD sequence appears to possess two rearrangement sites that are separated by at least ≈6.5 kbp of DNA (Fig. 1). A 3′ NIARD event appears to be more common than a 5′ event (13 3′ events compared to 4 5′ events in this analysis) and only the 3′ site is available for S_α rearrangements. Rearrangements involving the 3′ site may occur before the 5′ site becomes available for additional rearrangement events. This latter conclusion is based on our observation that all myelomas displaying 5′ NIARD rearrangements also possess 3′ NIARD rearrangements but many instances of 3′ events are seen without 5′ events.

Our findings strongly argue that the germ-line NIARD sequence and probably most NIARD rearrangements occur outside the C_H gene cluster. The former conclusion is strongly supported by the fact that the only *Eco*RI fragment in the ≈200 kbp of DNA of the BALB/c C_H cluster that is large enough to possibly correspond to the germ-line NIARD *Eco*RI fragment contains the γ2a and ε genes (9, 10, 23). The restriction map of this γ2a–ε-containing *Eco*RI fragment (23) and the NIARD *Eco*RI map (see Fig. 1) are quite dissimilar. Aside from this compelling argument and our results with MPC-11 DNA, the existence of a germ-line NIARD band in J558 DNA, from which all the C_H genes except for α are deleted (21), is also in complete agreement with the non-C_H-associated nature of this sequence. Our inability to discern any clear-cut correspondences between the sizes of restriction fragments bearing NIARD rearrangements and any C_H genes or C_H S regions in a variety of plasmacytomas persuasively suggests to us that except for S_α most NIARD events occur outside the boundaries of the C_H gene cluster. This conclusion is also strongly supported by our extensive observations with MPC-11 DNA. Because NIARD maps outside the C_H gene cluster, the 5′ NIARD event that occurs in J558 and its presumably analogous counterparts in PC 3609, PC 3612, and PC 4050 are also most simply explained by at least one rearrangement event that originally occurred outside the boundaries of the C_H genes.

We have no direct evidence that NIARD sequences originate from outside the V_H gene family. However, because V_H genes have been found to display a high degree of genetic polymorphism in their flanking sequences (ref. 26 and unpublished results), the apparent lack of restriction site polymorphisms

around the germ-line NIARD sequence in several different inbred mouse strains (NZB, BALB/c, Al/N, and C57BL/6J) (Table 1 and data not shown) suggest that this sequence is not close to the V_H gene families.

Implications for NIARD Rearrangements in Allelic Exclusion. NIARD rearrangements result in the inactivation of α genes in the J558 and TEPC 601 myelomas. We have no direct evidence whether NIARD sequences are expressed or contain transcription promoter sites. Previous results obtained with J558 polyadenylylated heterogeneous nuclear RNA and an α cDNA clone have revealed only one predominant, high molecular weight precursor for the cytoplasmic α heavy chain mRNA (27), suggesting that a NIARD rearrangement does not transcriptionally activate the C_α gene. One could argue that NIARD events contribute to a portion of the antibody allelic exclusion phenomenon. However, because we have not observed a NIARD event in eight IgA-producing hybridomas, NIARD's participation in allelic exclusion mechanisms would thus far appear to be a delayed or late event in the life span of a plasma cell.

Potential Involvement of NIARD in Chromosomal Translocations. An alternative hypothesis to explain the types of cells in which NIARD rearrangements occur (i.e., myelomas but not hybridomas) is that these rearrangements are associated with cellular transformation. Specific chromosomal translocation events have been observed in mouse myelomas and lymphomas (28). A portion of chromosome 15 specifically translocates to chromosome 6 or chromosome 12, which are known to harbor the κ and heavy chain immunoglobulin gene families, respectively (29, 30). One could raise the speculative idea that a NIARD rearrangement event is the result of such a chromosomal translocation. This notion would concur with our findings that (*i*) NIARD events appear to occur predominantly outside the C_H locus and (*ii*) no additional NIARD events were observed in the lymphocyte genomes of eight recently derived IgA-producing hybridomas. We hope that studies designed to assess the state of NIARD in non-Ig-producing lymphomas and the chromosomal origins of both NIARD and NIARD rearranging sequences will shed some light on this interesting notion.

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