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Murine lambda gene rearrangements: the stochastic model prevails over the ordered model

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The ontogeny of the immunoglobulin (Ig) gene rearrangement in mammalian B cells seems to be ordered. Heavy chain gene segments rearrange first, followed by light chain gene segments, κ before λ. The genomic organization of murine λ locus does not preclude the simultaneous expression of two subtypes from the same chromosome. In order to distinguish between an ordered and a stochastic model of rearrangement, a panel of 67 B cell hybridomas secreting either λ1, λ2, λ3 or λx (recently described) were analysed for VλJλ rearrangements. The results show that in 97% of cases, a single rearrangement occurred, favouring the stochastic model over the ordered one. Strikingly, the possibility of having a productive rearrangement if the first try results in an aberrant one is rare. We propose therefore, that the λ Ig is not necessarily required to ensure allelic and subtypic exclusion mechanisms. Moreover, in 97% of the cases, at least one κ allele is rearranged. Furthermore, the RS recombination has been detected in 77% of the cases. This suggests that, although the stimulation of κ precedes that of λ locus, the RS recombination acts as a transacting albeit dispensable λ activator.

Key words: allelic and subtypic exclusion/B cell differentiation/lambda locus/RS recombination

Introduction

To conserve antibody monospecificity, B cells must develop isotypic and allelic exclusion mechanisms (Pernis *et al.*, 1965; Cebra *et al.*, 1966). A single heavy (H) chain from the two potential H chain alleles, and a single light (L) chain from the two potential light chain alleles (κ or λ loci) will be expressed in a cell, thus producing a single Ig molecule. Concerning the first rearrangement events of Ig genes, it is now established that the ordered regulated model (reviewed by Yancopoulos and Alt, 1986) prevails over the stochastic models (Coleclough *et al.*, 1983; Claverie and Langman, 1984). The H chain genes rearrange first, followed by the light ones (H → L). Furthermore, the expressed membrane-bound form of μ chain (μm) which signals cessation of H chain locus rearrangement has been shown to activate rearrangement of the κ locus (Alt *et al.*, 1982; Alt *et al.*, 1984; Reth *et al.*, 1985; Iglesias *et al.*, 1987; Nussenzweig *et al.*, 1987; Reth *et al.*, 1987; Mantz *et al.*, 1988), thus cumulating positive and negative functions. With respect to the κ/λ isotypic exclusion, there is a considerable body of evidence for an ordered regulated model in which κ is expressed before λ (κ→λ). Firstly, most of κ-expressing B cells retain their λ loci in the germline configuration while most of λ expressing B cells have non-functional rearrangements at the κ locus (κ^-/κ^-) (Alt *et al.*, 1980; Hieter *et al.*, 1981; Durdik *et al.*, 1984; Siminovitch *et al.*, 1985). Furthermore, the few Abelson murine leukemia virus (A-Mulv) lines that progress to λ expression when propagated in culture, rearrange κ first and then start to rearrange λ (Persiani *et al.*, 1987; Müller and Reth, 1988). The RS recombination, described in the mouse by Durdik *et al.* (1984), supports the ordered regulated model and is thought to be involved in λ locus activation (Persiani *et al.*, 1987; Müller and Reth, 1988). It consists of the deletion of part or all of the Jκ−Cκ locus by a recombination event between the RS sequence located downstream of the Cκ segment and an heptamer nonamer signal located upstream of either Cκ or Jκ segments. An homologous element has also been found in the human κ locus, termed KDE (Siminovitch *et al.*, 1985). Thus, the κ/λ isotypic exclusion can be explained by an ordered model and H and κ allelic exclusions are mainly ensured through feedback control by the products, μm chain and complete Ig, respectively.

The genomic organization of the murine λ locus, however, does not preclude the simultaneous expression of two subtypes from the same chromosome. Four different chains can be obtained from the λ gene segments ordered on chromosome 16 as follows: Vλ2 Vλx Jλ2 Cλ2 Vλ1 Jλ3 Cλ3 Jλ1 Cλ1 (Storb *et al.*, 1988; Carson *et al.*, 1989). The λ1 chain is obtained from Vλ1 Jλ1 rearrangement, λ2 from Vλ2 Jλ2, λ3 from Vλ1 Jλ3 and λx from Vλx Jλ2. To produce a single Ig, B cells must therefore ensure 'subtypic exclusion' in addition to allelic exclusion. To understand further the subtypic exclusion mechanisms, two models of rearrangement were considered: an ordered model and a stochastic model. The aim of this study was to distinguish between the ordered model that predicts that one of the different subtypes systematically rearranges before the others, and a stochastic model that predicts no ordered rearrangement but a random choice of one λ subtype. The analysis of 67 λ hybridomas expressing one of the four chains shows that, in 97% of cases, only the productive rearrangement is detectable for each subtype. Moreover, in 77% of these hybridomas, RS recombinations occurred on one or two κ alleles while in 23% of the cases, no RS recombination was detected. Taken together, these results suggest a transpositive, albeit dispensable λ activation mechanism by RS, that allows a stochastic choice of rearrangement of the different λ gene segments.

Results

Lambda subtype rearrangements

The configuration of the λ loci in a large number of λ hybridomas was analysed by Southern blot assay (see Materials and methods). Four different probes were used

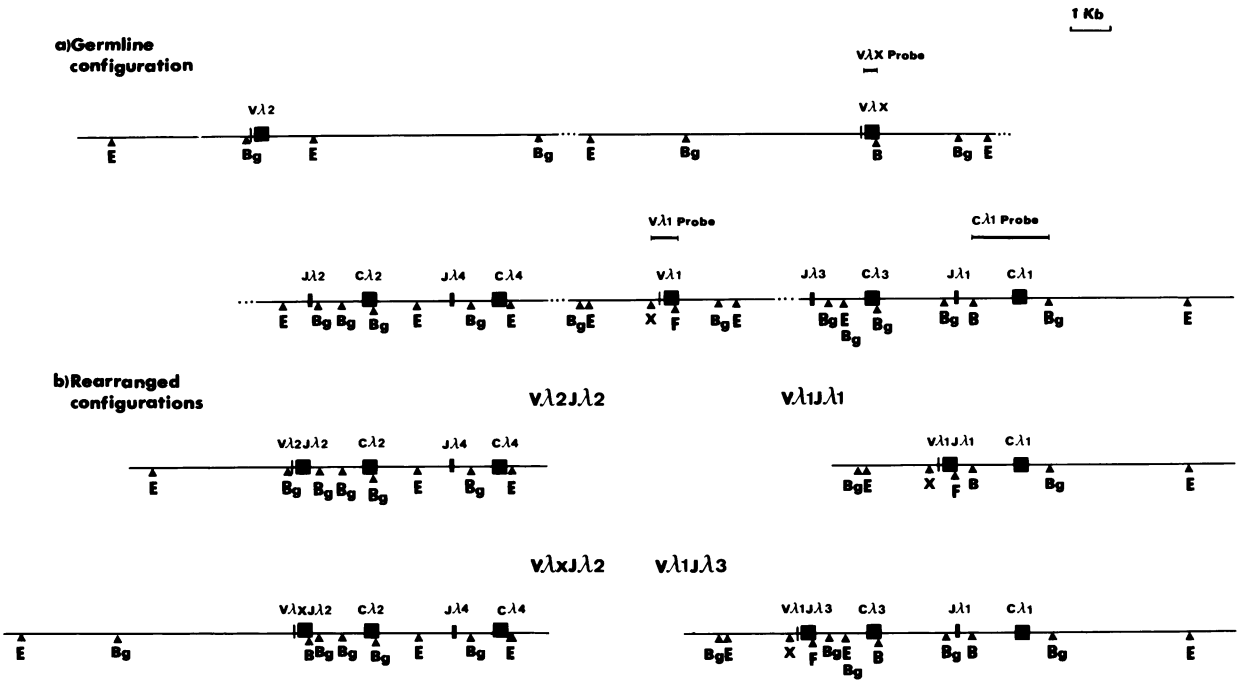

Fig. 1. Restriction enzyme maps of the murine λ locus: (**a**) Germline configuration: the λ probes used are indicated except Vλx Cλ2. (**b**) Rearranged configurations: the genomic organization allows simultaneous recombinations of Vλ1 to Jλ1 or Jλ3 and Vλ2 or Vλx to Jλ2 on the same chromosome. Vλx is deleted during Vλ2 to Jλ2 rearrangement, and Jλ3−Cλ3 are deleted during Vλ1 to Jλ1 rearrangement, while Jλ4−Cλ4 is a pseudogene. Adapted from Blomberg *et al.* (1982); Bernard *et al.* (1978); Bothwell *et al.* (1981); Arp *et al.* (1982); Selsing *et al.* (1982) and from our data. (E, *Eco*RI; B, *Bam*HI; Bg, *Bgl*II; X, *Xba*I; H, *Hin*dIII; K, *Knp*I; F, *Fok*I).

to identify and differentiate each λ subtype rearrangement (see Figure 1). The Vλ1 probe hybridizes both with Vλ1 and Vλ2 gene segments (>93% identity at the nucleotide level), the Vλx probe hybridizes specifically with the Vλx gene segment, the Vλx Cλ2 probe hybridizes both with the Cλ2 and Cλ3 gene segments (96% identity at the nucleotide level) and with Vλx gene segment, and the Cλ1 probe hybridizes specifically with the Cλ1 gene segment (Bernard *et al.*, 1978; Selsing *et al.*, 1982; Sanchez *et al.*, 1987b).

To identify joins involving Vλ1 and Vλ2 segments, we hybridized the Vλ1 probe to electrophoresed *Bgl*II digested BALB/c DNA (Figure 2A). Additional restriction enzyme fragments (REF) to those of the fusion partner (SP2/0) can be detected: a 4.5 kb band (Figure 2A lanes A3 to D4 and 1E106), a 0.8 kb band (Figure 2A lanes B23 to B15) and a 2.6 kb band (Figure 2A, lanes C1 to 1E72 and 3E123), corresponding respectively to the Vλ1 Jλ1, Vλ2 Jλ2 and Vλ1 Jλ3 rearrangements (see Figure 1). Except for 1E106 and 3E123, only one additional band is observed which must therefore correspond to the functional rearrangement. Because Vλ1 probe hybridizes with Vλ1 and Vλ2, we verified the identity of the observed rearrangements using other probes. The Vλ1 Jλ1 REF is confirmed using the Cλ1 probe on *Bgl*II digests (Figure 2C lanes A3 to D4 and 1E106). The Vλ2 Jλ2 rearrangement is confirmed by the presence of the additional 6.3 kb REF on *Eco*RI digests by probing with Vλ1 and Vλx Cλ2 (see asterisk on Figure 2D and Figure 2E, lanes B23 to B15). The Vλ1 Jλ3 rearrangement is confirmed by the additional 2.5 kb band on *Eco*RI digests by probing with Vλ1 (Figure 2D, lanes C1 to 1E72 and 3E123).

To identify the Vλx Cλ2 junction, the Vλx probe was used on *Bgl*II digested DNAs. A single additional 4.8 kb band was detected in λx producing hybridomas (Figure 2B, lanes 2E120 to 3E123). These rearrangements are confirmed, by probing with Vλx Cλ2 on *Eco*RI digests, by the presence of one 10 kb band (Figure 2E, lanes 2E120 to 3E123).

Since the utilization of Vλ1 and Vλx probes allows the identification of all Vλ Jλ junctions, the DNAs of 67 hybridomas were therefore assayed with *Bgl*II restriction enzyme (not shown). Of the 67 hybridomas (26 λ1, 11 λ2, 11 λ3 and 19 λx), 65 (97%) show only one rearrangment corresponding to the secreted protein subtype detected with RIA and IEF methods (not shown, Sanchez *et al.*, 1987a), and 2 (3%) show two rearrangements (1E106 and 3E123 described above). Moreover, no additional bands corresponding to the rare or theoretical Vλ2 Jλ3, Vλ2 Jλ1, Vλx Jλ3 and Vλx Jλ1 joins were seen with either probe (Elliot *et al.*, 1982; Reilly *et al.*, 1984; Weiss *et al.*, 1985). These results show that the majority of the λ-expressing B-cells studied have proceeded to a single type rearrangement, indicating that the stochastic model prevails over the ordered one.

RS recombination analysis

Since RS recombination has only been described in a small number of cases (Durdik *et al.*, 1984; Persiani *et al.*, 1987; Müller and Reth, 1988), a large panel of B cell hybridomas expressing one of the four λ chains were examined by Southern blot using the RS 0.8 probe (see Materials and methods). *Eco*RI and *Bgl*II digested DNAs from BALB/c liver, SP2/0, and some hybridomas are shown in Figure 3. DNA digests from the liver gave an *Eco*RI fragment of about 6 kb and a *Bgl*II fragment of about 9 kb, while DNA from SP2/0 gave an *Eco*RI additional fragment of about 12.5 kb and a *Bgl*II additional fragment of about 4.4 kb (Figure 3A and B). Depending on the hybridomas, zero, one, or two additional bands, corresponding to RS recombination event on zero, one, or two κ alleles, can be observed (see Figure

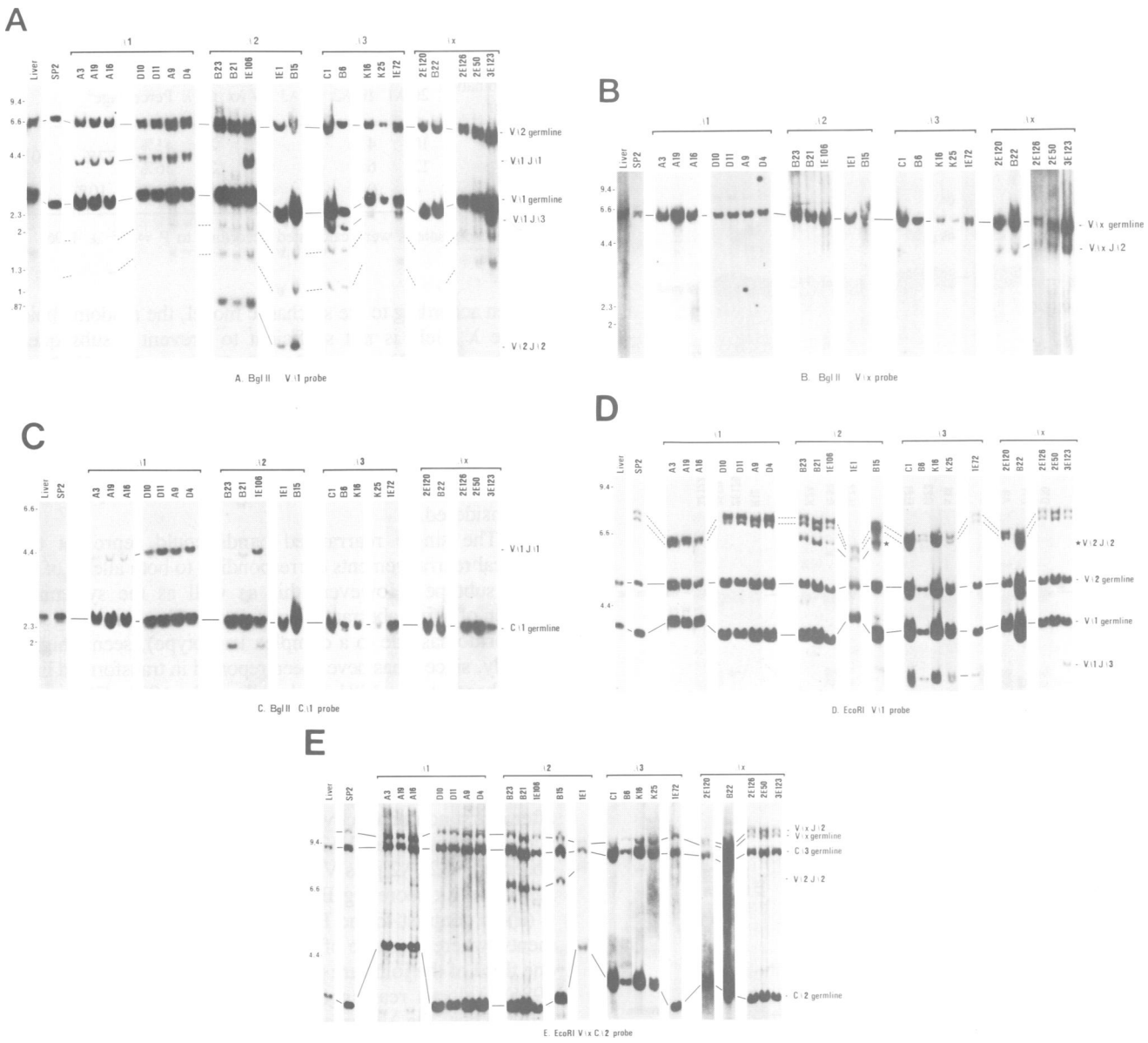

Fig. 2. Southern blot analysis of DNAs from representative λ secreting B cell hybridomas: *Bgl*II-digested DNAs hybridized with the Vλ1 (**A**), Vλx (**B**) and Cλ1 (**C**) probes, and *Eco*RI-digested DNAs hybridized with the Vλ1 (**D**) and Vλx Cλ2 (**E**) probes. Lanes designated SP2 represent DNA from the fusion partner (SP2/0) used to prepare these hybridomas. Lanes designated liver represent liver DNA from BALB/c. Germline and rearranged gene segments are indicated by solid lines. Additional bands from the fusion partner are indicated in dashed lines. Some additional bands are seen with some probes for B23 and 1E106 hybridomas the significance of which remains unclear.

3A and B). Figure 3A and B show that some differences are obtained in the number of rearranged alleles for a given hybridoma between *Eco*RI and *Bgl*II digested DNAs (e.g. lanes D11, K18, 1E106). Therefore, in order to search for undetectable *Eco*RI or *Bgl*II digested rearranged bands, *Bam*HI digested DNAs were also analysed (see Figure 3C). This third digestion confirmed the results obtained with either *Eco*RI or *Bgl*II digested DNAs except in a few cases (e.g. B21). A panel of 66 hybridomas were therefore analysed with the three enzymes. A summary of the results for RS recombinations is indicated in Table I. The final RS recombination number for each hybridoma corresponds to the highest RS recombination number obtained with *Eco*RI, *Bgl*II or *Bam*HI digested DNAs. The results (77% with one or two recombination events) suggest a good correlation between λ locus activation and RS recombination. To investigate further the configuration of the κ locus of the hybridomas presenting no RS recombination, *Eco*RI, *Bgl*II, and *Bam*HI digested DNAs were reprobed with the Jκ probe (not shown). Results show that, of the fifteen tested, ten clearly have one or two Jκ recombinations, two no Jκ recombination and three remain uncertain. The ordered mechanism favouring κ over λ rearrangements seems therefore to be respected.

Discussion

By analogy with the ordered rearrangement model H→κ→λ, that governs κ/λ isotypic exclusion, one can ask if an ordered model also controls the different λ subtype rearrangements, and thus, the λ subtypic exclusion. Such a model predicts that one of the different λ subtypes systematically rearranges

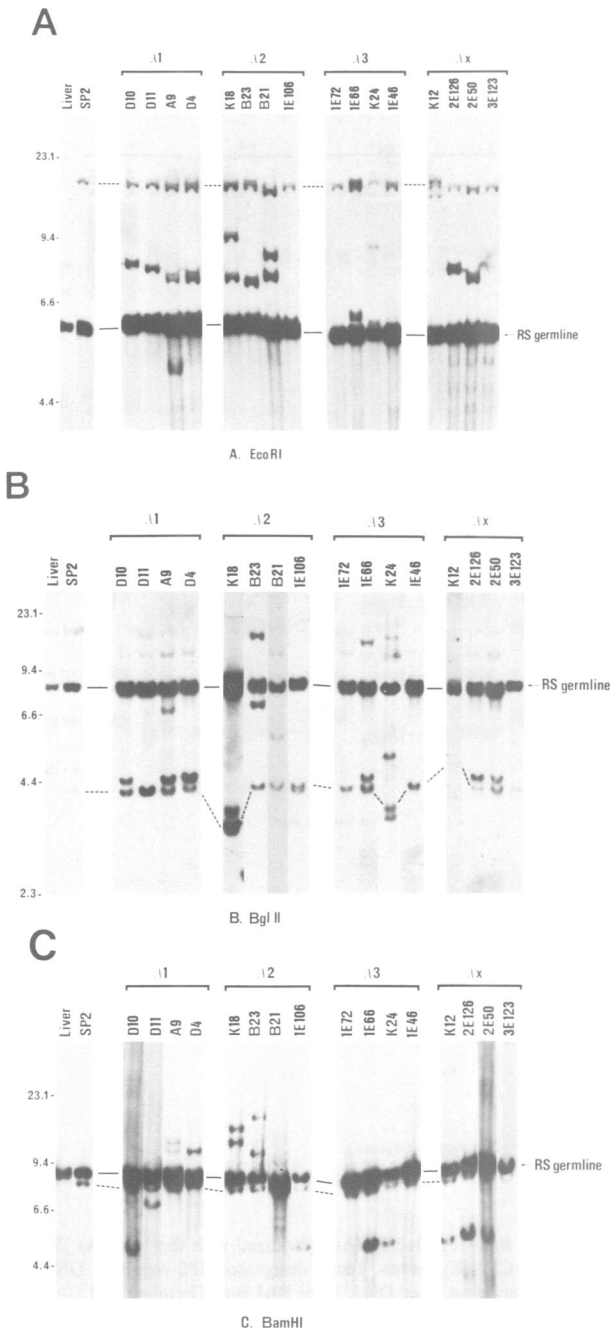

Fig. 3. Southern blot analysis of DNAs from some representative λ secreting B cell hybridomas: *Eco*RI (**A**), *Bgl*II (**B**) and *Bam*HI (**C**) digested DNAs hybridized with the RS 0.8 probe. Lanes designated SP2 represent DNA from the fusion partner (SP2/0) used to prepare these hybridomas. Lanes designated liver represent liver DNA from BALB/c. Germline RS band is indicated by solid lines. Additional bands from the controls are indicated in dashed lines.

first, and furthermore, systematically implies more than one rearrangement for all the other subtypes, corresponding to a non-random distribution of non-productive rearrangements. Another option is a stochastic model, where the choice of the λ subtype rearrangement is random. Such a model predicts a random distribution of the non-productive rearrangements. Our results allow us to eliminate the ordered model, since of the 67 λ hybridomas analysed, only two (1E106, 3E123) showed one non-productive rearrangement.

Table I. RS recombination events in the λ B cells

Final RS recombination number	λ hybridomas				Total		
	26 λ1	10 λ2	11 λ3	19 λx	66 λ	Percentage[a]	
2 RS	10	4	8	5	27	41%	77% ± 10%
1 RS	12	6	0	6	24	36%	
0 RS	4	0	3	8	15	23% ± 10%	

[a]Standard deviations were calculated according to $P = p° \pm 1.96 \sqrt{p° q°/n}$.

Even according to the stochastic model, the random choice of one λ allele is not sufficient to prevent a subsequent rearrangement. However, the drastic proportion of λ B cell hybridomas presenting a single rearrangement (97%) is especially significant. This means that very few B cells proceeded to a productive join after a first non-productive one. If one considers that the rearrangement probability is the same whatever the λ sublocus, three main options can be considered.

(i) The single rearranged bands could represent two identical rearrangements corresponding to both alleles of the same subtype. However, this as well as the systematic deletion of a first aberrant rearrangement (that cannot be seen in hybridomas due to a complex karyotype), seems highly unlikely, since it has never been reported in transformed lines (Blomberg *et al.*, 1981 Bothwell *et al.*, 1981; Wu *et al.*, 1982; Reilly *et al.*, 1984; Müller *et al.*, 1988). Moreover, in the Cκ locus, when the first Vκ−Jκ join is non-productive, the second Cκ allele uses generally another Vκ to form the second join. By analogy, it is conceivable that after a first non-productive Vλ Jλ2 join, the second Jλ2 Cλ2 allele could use either Vλ2 or Vλx. However, we have never observed Vλ2 Jλ2 plus Vλx Jλ2 rearrangements, neither in λ2 nor in λx secreting B cells.

(ii) In contrast to the H and κ gene segment rearrangements, where, because of the reading frame, approximately one third of the joins are productive (Yancopoulos and Alt, 1986), λ locus rearrangements would be rarely prone to error. Thus, the λ rearrangement mechanism would use a distinct correction mechanism (addition or withdrawal of nucleotides to restore the reading frame), or especially reliable recombination sequences, or a different recombinase activity. But the fact that the rearrangement mechanisms were found to be highly conserved between B and T cells (Yancopoulos and Alt, 1985; Alt *et al.*, 1986; Schuler *et al.*, 1986; Yancopoulos *et al.*, 1986; Lieber *et al.*, 1987; Malynn *et al.*, 1988; Blackwell *et al.*, 1989) strongly suggests that the rearrangement mechanism as well as the transacting recombination factors are the same for all of the Ig loci of B cells.

(iii) After a first unsuccessful λ rearrangement, B-cells would reach a developmental stage where they become unable to subsequently rearrange another λ allele. This is in keeping with previous results (Lieber *et al.*, 1987) with extrachromosomal DNA substrates, showing that the recombination activity decreases during B lymphoid development, reaching undetectable levels at the mature B cell stage.

We favour this third option, and suggest that the λ Ig is not necessary to stop the recombination process, although a negative feed-back by the rearrangement product has been

proposed, according to recent reports of transgenic mice expressing a λ transgene (Neuberger *et al.*, 1989; Hagman *et al.*, 1989). Our hypothesis is supported by two lines of evidence. Firstly, in the transfection experiments described above (Lieber *et al.*, 1987) one mature Ig positive B-cell line retained significant recombinase activity, indicating that the presence of surface Ig is not by itself sufficient to shut off V(D)J recombination activity. Secondly, in the chicken, the vast majority of B cells contains one functionally rearranged λ allele and one unrearranged allele (Reynaud *et al.*, 1985; Thompson and Nieman, 1987). However, only one third of the VJ joins are in frame in the B cells from embryonic days 10–12 of development, suggesting that cells which contain a non-functional rearrangement on one allele cannot rearrange the other. Thus, B cells may present a VJ recombinase activity for only a short time (McCormack *et al.*, 1989).

We did not consider in this work a 'pure stochastic model' where the probability of initiation of rearrangements at the λ locus is particularly low, because the ordered scheme H → L, implies that the λ light chains are also activated. The nature of this activation signal remains to be determined, but the RS recombination may be a possible candidate. Indeed, three main hypotheses have been considered (Durdik *et al.*, 1984; Persiani *et al.*, 1987; Müller *et al.*, 1988): (i) there is only a chance correlation between RS expression and λ locus activation without a cause–effect relation, (ii) the RS recombination allows the activation of a *trans*-positive factor (in this case, only one RS recombination is sufficient), (iii) the RS recombination inhibits a *trans*-repressing factor (implying the deletion of the repressor on both alleles). However, RS recombination has only been studied in a few independent murine λ-expressing B cell clones (Durdik *et al.*, 1984; Persiani *et al.*, 1987; Müller *et al.*, 1988). Our results show 77% of hybridomas with one or two RS recombination events. This suggests a good correlation between RS recombination and λ locus activation. A rather high percentage (23%), however, shows no RS recombination. This result could be overrated since loss of RS alleles cannot be excluded in the hybridomas. In the majority of cases where no RS recombination can be detected, there is at least one Jκ rearrangement implicating that this percentage (23%) cannot only be explained by this fact. We therefore conclude that these hybridomas proceeded to λ locus rearrangement without a RS recombination event. On the other hand, 36% of λ B cell hybridomas show only one RS recombination. This could imply a *trans*-positive activation, because a putative repressor should be deleted from both alleles to allow the activation of the λ locus. Taken together, these results suggest the occurrence of a *trans*-positive RS factor, active but dispensable. This observation of a moderate action of the RS signal, shows some similarities with that of μm. Thus, though it has been shown that μm activated the κ locus, a recent report on SCID mice showed the possibility of circumventing this process since in one case, progression to the stage of κ light chain gene rearrangement did not require expression of μ chain (Blackwell *et al.*, 1989). This would suggest that RS recombination as well as μm signal, represent a step in an activation cascade.

In conclusion, our results support a stochastic model in which the λ allelic and subtypic exclusions are not necessarily ensured by the production of a λ positive Ig, preventing subsequent rearrangement. In contrast to the H and κ chains, the probability of seeing a non-productive rearrangement is very low, suggesting that B cells which functionally or non-functionally rearrange λ genes cannot proceed to another rearrangement. It is conceivable that such B cells reach a developmental stage where they become unable to retain recombinase activity. This observation is in keeping with studies in chicken (Reynaud *et al.*, 1985; Thompson and Nieman, 1987; McCormack *et al.*, 1989). Moreover, the recent description of primitive vertebrate Ig light chains, shows that the amino acid sequences are closely related to mammalian λ chains (Shamblott *et al.*, 1989). Thus, it can be speculated that the λ chain is the primitive partner of the heavy chain. The κ system would subsequently have differentiated, developing a set of mechanisms that allows it to be expressed before λ. The RS recombination could belong to this set, as an activator, albeit dispensable, that does not preclude a direct stimulation of the λ system. We predict therefore that our observations will be similar for other mammalian λ systems especially in humans.

Materials and methods

λ B cell hybridomas

Spleen cells from BALB/c mice were fused with SP2/0, 3 days after a single injection of 10 μg of rabbit anti-λ antibodies coupled to LPS, as described previously (Sanchez *et al.*, 1987a). P1 is a X63 Ag/8 hybrid and 1.5B6 and 6-15E8 are derived from the SJA strain. B cell hybridomas secreting a single λ Ig [detected by RIA and SDS PAGE/IEF methods (Sanchez *et al.*, 1987a)] were then selected for the analysis (twenty six λ1; eleven λ2; eleven λ3; nineteen λx). For each subtype, hybridomas were obtained from at least three individual mice.

Southern blot analysis

*Bgl*II, *Eco*RI, or *Bam*HI digested DNAs from each λ subtype-expressing B cell hybridoma were performed. Roughly 12 μg of each DNA sample were electrophoresed and analysed by Southern blotting techniques (Southern, 1975). Roughly 100 ng of probe was labelled with an activity of about 10^7 cpm. Following hybridization, Hybond filters (Amersham) were washed in 0.1% SDS, 15 mM NaCl, 1.5 mM sodium citrate, pH 7, at 65°C for 30 min.

Probes and restriction maps

The RS specific probe, generously given by M.Reth was the 0.8 kb *Sau*3A fragment from the RS region (Persiani *et al.*, 1987). The Jκ probe was the *Hin*dIII–*Xba*I fragment from the Jκ region (Max *et al.*, 1979). The Vλ1 probe was the 0.6 kb *Xba*I–*Fok*I fragment from the Vλ1 region (Bernard *et al.*, 1978). The Vλx probe was the 0.3 kb *Hin*dIII–*Bam*HI fragment from the Vλx region of a Vλx Cλ2 cDNA clone (Sanchez *et al.*, 1987b). The Cλ1 probe was the 1.8 kb *Bam*HI–*Bgl*II fragment from the Cλ1 region (Bernard *et al.*, 1978). The Vλx Cλ2 probe was the 0.6 kb *Bam*HI–*Bam*HI fragment from the Cλ2 region of a Vλx Cλ2 cDNA clone (Sanchez *et al.*, 1987b). The λ probes used are indicated in Figure 1, except the Vλx Cλ2 probe.

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