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Gene-conversion in rabbit B-cell ontogeny and during immune responses in splenic germinal centers☆

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Abstract

Combinatorial diversity is limited in rabbits because only a few V_H genes rearrange. Most diversification of the primary repertoire is generated by somatic hypermutation and gene conversion-like changes of rearranged V_H in B cells that migrate to appendix and other gut associated lymphoid tissues (GALT) of young rabbits. The changes are referred to as gene conversion-like because the non-reciprocal nature of the alterations introduced has not yet been demonstrated. There are many similarities between rabbits and chickens in how their B cells develop and diversify their repertoires. However, although the majority of rabbit B cells may have rearranged and diversified their V genes early in life, some B cells in adult rabbits have rearranged V_H sequences that are identical or nearly identical to germline sequences. We found these cells in splenic germinal centers (GC) on days 7 and 10 after immunization of normal adult rabbits with DNP-BGG. By day 15, all rearranged V_H sequences were diversified. We find an overall pattern of splenic precursor cells whose germline or near germline sequences change both by gene conversion and point mutations during early divisions and mainly by point mutations during later divisions. These events, in parallel with diversification of light chain sequences, may produce the diverse combining sites that serve as substrates for further affinity maturation by selection either within GC or later among emigrant cells in sites such as bone marrow. Some of the sequences altered by gene conversion in splenic germinal centers may also produce new members of the B-cell repertoire in adult rabbits comparable to those produced in GALT of neonatal rabbits.

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1. Introduction

Studies in the rabbit have historically yielded new insights into a variety of aspects of the genetics, development and functions of the immune system. These include: allelic exclusion, recombination between V_H and C_H genes, allotype suppression, idiotypes, and network interactions in immune regulation (reviewed in Mage, 1998). This tradition continues with the observations of repertoire development and expressed V_H gene diversification discussed below.

2. Background

2.1. Rabbit Ig genes

Fig. 1 shows a diagrammatic summary of the genes that can contribute to antibody heavy and light chains of the rabbit. Overall, the organization is similar to that described for mouse and man (Shimizu et al., 1982; Mainville et al., 1996; Matsuda and Honjo, 1996). Notable differences are, for the heavy chain locus, the presence of a single gene for the constant region of IgG and multiple copies of genes for the constant region of IgA (13 of which 12 are probably functional) (reviewed in Mage, 1998). In addition, although the V_H gene organization is comparable to other species, there is preferential rearrangement and expansion of B lymphocytes that utilize the first gene in the locus, V_H1, that is nearest to the D_H and J_H genes (reviewed in Knight, 1992). The V_Ha allotypes (a1, a2 and a3), allelic forms of the V_H1 gene product, were characterized many years ago (reviewed in Mage et al., 1984). It is because the rearranged V_H1 gene is expressed on the majority of B lymphocytes, that these V_H region allotypes were identifiable (Knight and Becker, 1990).

There are also allelic allotypic forms of the rabbit kappa light chain (b4, b5, b6, and b9) due to multiple amino acid differences in the constant regions. At some point in evolution, an ancestor of the rabbit and other lagomorphs underwent a duplication in the kappa locus leading to the two different Cκ genes about 1 Mb apart (Hole et al., 1991) kappa 1, the major expressed type of kappa light chains and kappa 2, expressed by only a minor subset of cells. An exception is in mutant Basilea rabbits (Kelus and Weiss, 1977) where because of a defect in the kappa 1 acceptor site for Jκ to Cκ mRNA splicing (Lamoyi and Mage, 1985), the mutants express elevated levels of both kappa 2 and lambda light chains.

2.2. Rabbit B cell development – the role of gut associated lymphoid tissue (GALT) in primary repertoire development

After an important role for the chicken bursa of Fabricius in development of humoral immunity of chickens was reported (Glick, 1956), the question of whether there was a

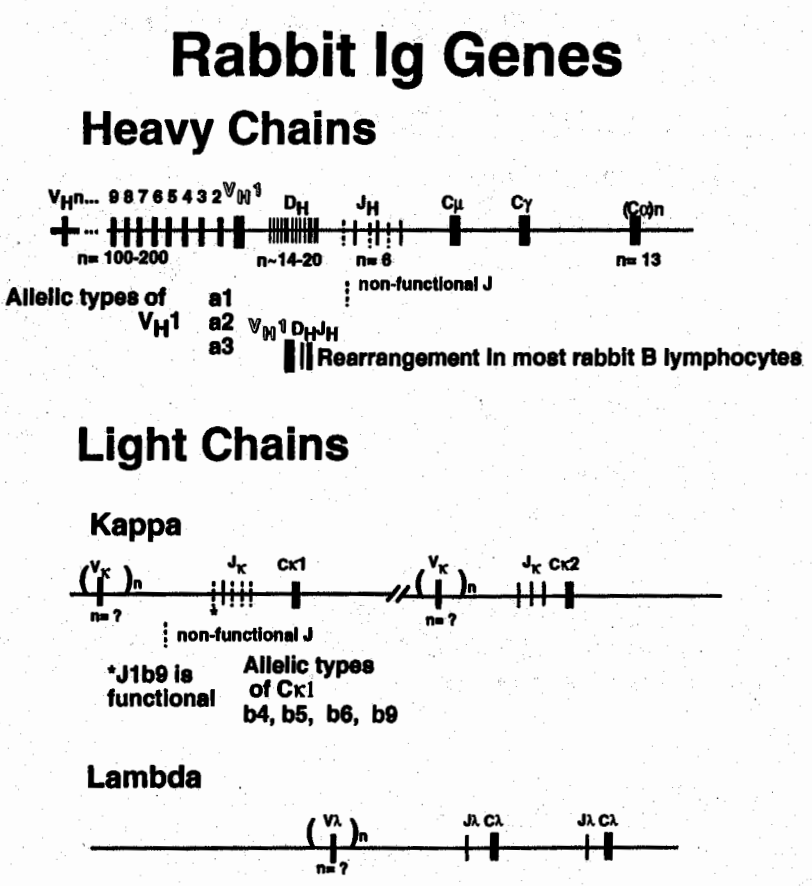

Fig. 1. Diagrammatic summary of rabbit immunoglobulin (Ig) genes at the heavy and light chain loci.

mammalian bursal equivalent was raised. In particular, the laboratory of Robert Good conducted a series of investigations of development of the immune system in chickens and rabbits. Archer et al. (1963) noted the remarkable histological similarity of bursal tissue to 'Peyer's patch-type' GALT such as the appendix of the rabbit. By 1968, an antibody deficiency syndrome had been produced in rabbits by neonatal removal of organized intestinal lymphoid tissue (Cooper et al., 1968; Perey et al., 1968) suggesting that 'Peyer's patch type lymphoepithelial tissue in rabbits represented the mammalian homologue of the bursa of Fabricius'. With the discovery of the role of bone marrow in the initial stages of B lymphocyte development, the idea that there was a mammalian bursal equivalent was set aside.

Bursectomized chickens were observed to make IgM but were nevertheless immunodeficient (Lerner et al., 1971). This was explained when it was shown that the chicken bursa was the site of diversification of rearranged V_H and V_L in already formed B-lymphocytes rather than the site of lymphocyte development from stem cells (Pink et al., 1985). The diversification of rearranged V_H and V_L in chickens was shown to occur by gene conversion (Reynaud et al., 1987; Thompson and Neiman, 1987; Reynaud et al., 1989) involving non-reciprocal exchange of sequence information utilizing pseudogenes

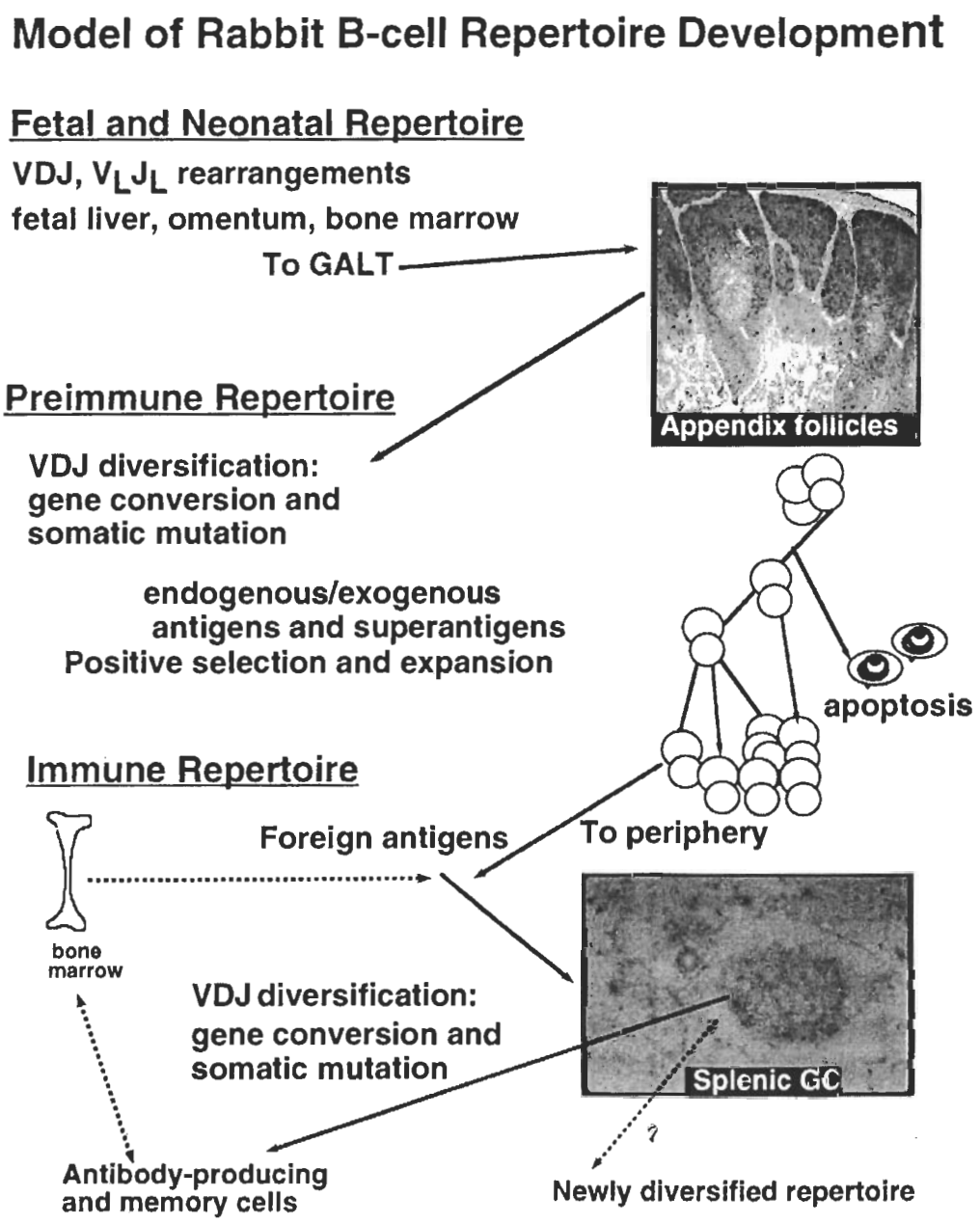

Fig. 2. A working model of B cell development and diversification in the rabbit.

upstream of the one functional rearranging V_H or V_L gene (Carlson et al., 1990). In rabbits, diversification that appears to be gene-conversion-like is observed although the non-reciprocal nature of the process has not been formally demonstrated.

Fig. 2 shows a current working model of the development of the B-cell repertoire in the rabbit. Particularly after the reports that combinatorial diversification of rabbit V_H was limited because of preferential rearrangement of the V_H1 (a-allotype-encoding) gene (Knight and Becker, 1990; Allegrucci et al., 1991) and that diversification of rearranged

$V_HD_HJ_H$ occurred by gene-conversion-like changes as well as somatic mutations (Becker and Knight, 1990; Roux et al., 1991), the idea that rabbit GALT may be a mammalian bursal homologue was revived (Weinstein et al., 1994a, b). The studies of Weinstein et al. (1994a, b) and subsequent studies outlined below showed that there is great similarity between how rabbit and chicken B cells develop and diversify their repertoires. In young rabbits, gene rearrangements and initial development of B lymphocytes occurs in sites such as bone marrow, fetal liver, omentum and newborn spleen, but an important site for B-cell repertoire diversification is the appendix and other organized GALT. Even removal of only the neonatal rabbit appendix impairs mucosal immune responses of young rabbits (Dasso and Howell, 1997). When neonatally appendectomized rabbits were immunized with ovalbumin (OVA) at 45–48 days of age, they showed a highly significant decrease in total IgA^+ and IgA^+ anti-OVA specific cells in the gut, lower total IgA, IgG as well as specific anti-OVA levels locally and systemically (for IgG anti-OVA) compared to sham operated controls. Weinstein et al. (1994a, b) showed that the rabbit's appendix develops after birth from having no organized B- and T-follicular regions to become a highly cellular collection of several hundred follicles with T cells mainly in inter-follicular regions and B-cell rich follicles containing light and dark zones. By 6 weeks of age, they found B cells in the appendix were proliferating and undergoing diversification of their rearranged V_H genes by gene conversion and hypermutation. Thus, there was not only structural homology (Archer et al., 1963) but functional similarity to the chicken bursa of Fabricius. Finally, Vajdy et al. (1998) wanted to determine the extent to which somatic diversification of V_H genes was dependent upon GALT in rabbits. They essentially repeated the 1968 experiments of the Good laboratory (Cooper et al., 1968; Perey et al., 1968) surgically excising from 1-day-old rabbits, the appendix and ileocecal junction in which the sacculus rotundus develops. At 3–5 weeks of age they also removed visible Peyer's patches of the small intestine. When only appendix was removed, the sequences of rearranged $V_H1D_HJ_H$ in 9-week-old rabbits' peripheral blood lymphocytes (PBL) were somewhat diversified. When appendix and sacculus rotundus were removed at birth and Peyer's patches at about 4 weeks of age, the rearranged $V_H1D_HJ_H$ of the 'GALT-less' rabbits had undergone little diversification at 10–12 weeks of age. Thus, at these early ages, GALT appeared to be a key site for development of a diverse repertoire. Limited diversity continued to be observed in PBL at 19 weeks of age. However, by 28 weeks, the young adult rabbits had extensive sequence diversity of the $V_H1D_HJ_H$ in their PBL. In addition to the important role that GALT played in repertoire development, the total numbers and percentages of B lymphocytes were lower in GALT-less rabbits. These results further supported the concept that rabbit GALT is a bursal homologue; primary development and expansion of B lymphocytes is dependent in part on intact GALT. The hypothesis was advanced that as in chickens, most repertoire development occurs in young rabbits with cells exiting GALT becoming the source of self renewing peripheral cells utilized in immune responses throughout life (Mage, 1993; Crane et al., 1993, 1996; Pospisil and Mage, 1998). The chronic depressed expression of a kappa or V_Ha allotype targeted by neonatal treatments with anti-allotype antibodies (allotype suppression) highlights the importance of the neonatal period in establishment of the rabbit's life-long repertoire (Mage, 1975). The gradual reappearance of B cells with the suppressed allotype (Mage, 1975) as well as our recent observations of B cells with germline

rearranged $V_HD_HJ_H$ in adult splenic germinal centers (Sehgal et al., 1998) indicate that some B lymphopoiesis probably occurs in adult rabbits.

The development of B cells with diverse $V_H1D_HJ_H$ sequences in GALT-less rabbits by 7 months of age may have occurred in residual GALT lymphoid aggregates that were not visible when Peyer's patches were removed at four weeks of age. In addition, B cells from the GALT-less rabbits appeared to colonize spleen and lymph nodes where germinal centers developed. We now know that even in normal adult rabbits there is diversification of rearranged V_H genes by gene conversion and hypermutation during specific immune responses in splenic germinal centers.

3. Immune repertoire

We asked whether somatic gene conversion, hypermutation or both occur in rabbit splenic germinal centers (GC) during the primary immune response to DNP coupled to bovine gamma globulin. The sequences of PCR-amplified rearranged V_H and $V\kappa$ were determined on single DNP^+ or Ki-67^+ B cells from DNP^+ GC collected at days 7, 10, and 15 of the anti-DNP response. Although the majority of rabbit B cells may have rearranged and diversified their V genes early in life, we found B cells in splenic germinal centers with V_H sequences that were identical or nearly identical to germline sequences on days 7 and 10 in adult rabbits (Sehgal et al., 1998). More than half of the sequences at days 7 and 10 were independent and unique but this decreased to about 1 in 10 at day 15 (Schiaffella et al., 1999). By day 15, all sequences were diversified, and large groups of clonally related sequences predominated. The overall pattern that is documented by these studies, is one of splenic precursor cells whose germline or near germline sequences changed both by gene conversion and point mutations during early divisions and mainly by point mutations during later divisions. Our preliminary data show that within the same expanding clonal populations, considerable diversification of light chain sequences occurred in parallel with the changes in the V_H sequences. These events may produce the diverse combining sites that serve as substrates for further affinity maturation by selection either within GC or later among emigrant cells in sites such as bone marrow.

4. Concluding remarks

Does some repertoire diversification by gene conversion persist in the adult rabbit's secondary lymphoid tissues? When it was observed that RAG gene expression was reactivated in germinal centers, the possibility that some new repertoire development occurred in the germinal center was raised by several authors (Han et al., 1996; reviewed in Tarlinton, 1997; Rajewsky, 1998). In mice the new receptors are mainly the result of replacement of light chains. However in rabbits, some of the rearranged V_H sequences altered by gene conversion may produce new members of the B-cell repertoire in adults comparable to those produced in GALT of young rabbits. The reappearance of gene conversion in rabbit splenic GCs provides an important example of re-expression of immature functions (neoteny).

5. Summary

Early in life, a repertoire of diverse B lymphocytes is generated in rabbit GALT. This is a source of responsive cells needed for the primary repertoire. During B-lymphocyte expansion in appendix and other GALT of young rabbits, diversity of limited numbers of rearranged V_H genes is increased somatically via a gene-conversion-like mechanism as well as somatic hypermutation. Although much of the repertoire of the rabbit may be generated in GALT early in life, some cells with properties of newly arisen B lymphocytes are found in germinal centers of the adult spleen. During specific immune responses in splenic germinal centers of adults, cells with germline or close-to-germline VDJ sequences undergo gene conversion-like changes. In clonally expanding cells, we generally find alterations by gene conversion, followed by point mutations. Within a germinal center, changes in V_H sequences are paralleled by changes in light chain sequences. These cells with diverse receptors may be the substrates for selection of high affinity receptors either in the germinal center or in sites of further development toward antibody production such as bone marrow.

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