Old Herborn University Seminar Monograph 6:
The ontogeny of the immune system.
Editors: Peter J. Heidt, Dan Holmberg, Volker Rusch, and Dirk van der Waaij.
Herborn Litterae, Herborn-Dill, Germany: 1-6 (2003).

ESTABLISHMENT AND MANAGEMENT OF B LYMPHOCYTE REPERTOIRES

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SUMMARY

This paper focuses on some aspects of the establishment and maintenance of antibody repertoires. We will discuss a developmental program where the perinatal B cell repertoire is established through i) genetic mechanisms imposing the expression of a highly connected, germline encoded idiotypic network ii) a limited somatic diversification process at this stage of ontogeny which guarantee the expression of these germline encoded properties iii) cellular selection favouring clones displaying these properties. In the adult similar genetic constraints appear to apply to the emergent repertoire of pre-B cells of the bone marrow. In contrast to the perinatal period, however, somatic diversification (e.g. N-sequence additions) at this stage is abundant and clones displaying high connectivity appears to be selected against.

INTRODUCTION

A central problem of immunology today concerns the development and control of lymphocyte repertoires. The lymphocyte repertoire consists of individual B lymphocytes expressing antigen receptors (surface or secreted 19), with V-regions generated through recombination of V, (D), and J gene segments during early stages of lymphocyte differentiation. As a consequence of understanding the genetic mechanisms responsible for the generation of 19 diversity, increasing interest has been directed to defining the mechanisms controlling the development and the maintenance of this diversity. By estimating the number of different V, D, J (for 19 heavy chain) and V and J (for light chain) gene segments present in the germline (*Tonegawa*, 1983), it is obvious that the number of potential combinations by far exceed the number of B lymphocytes that are present in a mouse at any time (*Jerne*, 1971). This observation rises the question of on which bases the V-regions represented in the periphery are selected. The rules and mechanisms mediating these processes concerning are in the focus of this review.

GERMLINE ENCODED CONNECTIVITY

It is well established that the B lymphocytes in perinatal and in adult individuals differ considerably with respect to specificity repertoires (*Silverstein* et al., 1963, *Klinman* and *Press*, 1975) and to V gene utilisation (*Yancopoulos* et al., 1984, *Perlmutter* et al., 1985, *Dildrop* et al., 1985). One important

difference relates to the degree of connectivity displayed by the immune system (IS) at different points of ontogeny. Connectivity, measured as mutual recognition between monoclonal IgM antibodies derived from B cells at different stages in ontogeny, has been demonstrated to be considerably higher in foetal liver (FL) and newborn (Nb) spleen as compared with adult (Ad) spleen (*Holmberg* et al., 1986, *Vakil* and *Kearney*, 1986). This V-region connectivity among B cells appears to result in part from the programmed expression of germline encoded V gene specificities and in part from a learning process based upon interactions between the developing B cell clones and different "self"-ligands.

Molecular analyses of B cell hybridomas displaying mutual reactivity have demonstrated the germline origin of the 19 receptors included in such a network of V-region interactions (*Carlsson* and *Holmberg*, 1990, *Carlsson* et al., 1991). Moreover, mechanisms mediating somatic diversification of the junctional region between the V_H, D, and J_H gene segments are minimised early in ontogeny. This is evident particularly for the addition of N-region nucleotides (*Alt* and *Baltimore*, 1982) which are rare in sequences of foetal and neonatal origin but abundant in sequences of adult origin (*Carlsson* and *Holmberg*, 1990, *Holmberg* et al., 1989, *Gu* et al., 1990, *Feeney*, 1990). As argued before, the limited somatic diversification of perinatal V-regions may guarantee the expression of germline encoded specificities in the early IS (*Holmberg* et al., 1989).

In parallel with the functional characteristics of high connectivity, the perinatal B cell repertoire expresses V_H genes in a non-random fashion. Thus, B cells of the FL and the neonatal spleen preferentially utilise V_H genes of the D-proximal V_H gene families (V_H7183, V_HQ52), whereas the repertoire expressed in the adult spleen shows no obvious bias in this respect (*Yancopoulos* et al., 1984, *Dildrop* et al., 1985, *Jeong* and *Teale*, 1988, *Freitas* et al., 1989). A non-random V gene utilisation may result from mechanisms favouring (or disfavouring) certain V gene segments during the process of V(D)J assembly at the early pre-B cell stage. Alternatively, certain V(D)J rearrangements may be intra- or inter-cellularly selected on the basis of their specificity. Evidence has accumulated during the last few years suggesting that mechanisms of both types contribute to the establishment of the mature B cell repertoire.

INTRINSIC RATES OF V(D)J REARRANGEMENTS

The first observations demonstrating a non-random utilisation of V_H genes in early B cells were derived from analyses of Abelson murine leukaemia virus (A-MuLV) transformed pre-B cell lines, which continuously undergo V_H to DJ_H rearrangements in culture (*Yancopoulos* et al., 1984). Together with similar analyses of FL hybridomas, these studies suggested that the observed bias in the foetal and perinatal B cell repertoires was a result of mechanistic constraints on the 19 gene assembly process, i.e. chromosomal positioning and accessibility to the recombination machinery (*Alt* et al., 1986, *Blackwell* et al., 1986). Clearly, V_H gene families positioned in the proximity of the D region of the IgH locus are preferentially utilised in the process of V_H to D-J_H rearrangements. This preference is most marked for one particular V_H gene of the

V_H7183 family, the V_H7183.1 gene segment (previously denoted 81 X), which in A-MuLV B cell lines is utilised in almost 30% of all rearrangements (*Yancopoulos* et al., 1984, *Reth* et al., 1986, *Lawler* et al., 1987). More recent analyses of V_H gene utilisation using PCR technology to amplify V_H7183 rearrangements from genomic DNA have confirmed these findings (*Carlsson* et al., 1992). Further support for a biased rearrangement machinery was evident by analysing the frequency with which the V_H7183.1 gene occurred in non-productive rearrangements. Non- productive rearrangements are presumable non-selectable and would therefore to a large extent reflect the rearrangement machinery. The analyses of non-productive V_H7183 rearrangements revealed that the relative frequency of V_H7183.1 rearrangements remains constant at about 70% of the total V_H7183 rearrangements, irrespective of organ localisation and the developmental stage (*Huetz* et al., 1992).

In conclusion, these data strongly suggest that mechanistic constraints exist favouring the rearrangement of this gene segment over the other members of the V_H7183 gene family. However, chromosomal positioning cannot be the only factor determining the frequency of individual V_H gene segment rearrangements. The V_H7183.8 gene segment (previously denoted V_HE4.Psi), which is more proximal to the D region in the BALB/c genome, rearranges with a lower frequency than the V_H7183.1 gene segment in AMuLV-transformed pre-B cell lines (*Yancopoulos* et al., 1984) and are rarely found in the V_H7183-DJ_H PCR libraries (*Carlsson* et al., 1992, *Huetz* et al., 1992).

POSITIVE SELECTION OF EARLY B CELL REPERTOIRES

Although mechanistic constraints on the V_H-D-J_H recombination machinery may favour the rearrangement of certain D-proximal V_H genes, cellular selection also appears to contribute to the establishment of the perinatal B cell repertoire. Evidence for positive selection has been obtained from sequencing V_HDJ_H joinings of genomic DNA from B cell populations in perinatal and adult life. Thus V_H7183 rearrangements of adult origin show expected frequencies of out-of-frame rearrangements, whereas essentially all V_H7183 rearrangements of perinatal origin are in-frame (*Carlsson* et al., 1992). Further support for positive selection of B cells in neonatal individuals is indicated by that approximately 30% of the V_H7183.1-DJ_H rearrangements derived from neonatal pre-B cells are productive, while >80% of the V_H7183.1-DJ_H rearrangements are productive in the neonatal B cells (*Huetz* et al., 1992). These observations constitute the first formal evidence for positive selection of precursor B cells during the perinatal period of an individual, and argues against that V_H7183.1 utilising pre-B cells are not able to clonally expand as suggested by *Decker* et al. (1991).

The expansion of B cells during the perinatal period is not a result of intrinsic properties of the cells produced at this point in life since adult B lymphocytes transferred to neonatal recipients behave like the endogenously produced B cells with respect to growth and persistence (*Thomas-Vaslin* et al., 1991). Furthermore, recent analysis of adult mice with severe combined immunodeficiency (SCID) suggest that the ontogenic program of V_H gene repertoires can be "replayed" if these mice are reconstituted with adult BM cells from normal donors. Thus, up to 2 weeks

after reconstitution, SCID mice display a V_H repertoire resembling that of normal, neonatal mice with a considerable part of the 7183.1- DJ_H rearrangements being productive. However, 8 weeks after reconstitution the recipient mice have established a V_H repertoire resembling the normal adult mice.

NEGATIVE SELECTION OF ADULT B CELL REPERTOIRES

In adult life, most mature B cells are believed to be produced from the differentiation of precursor cells in the bone marrow, rather than by division and clonal amplification of pre-existing peripheral B cells. Although the V_H utilisation in adult, peripheral B cell repertoires appears to roughly represent the germline gene complexity of each of the V_H gene families, in situ hybridisation studies show that the intrinsic biases of V_H gene rearrangements in the adult bone marrow are the same or similar to those observed during the perinatal period (*Freitas* et al., 1990). This is further supported by the fact that the ratio of V_H7183.1 to other V_H7183 genes observed among non-functional rearrangements is constant all through ontogeny as previously discussed (*Huetz* et al., 1992).

Similar to the perinatal situation, cellular selection appears to contribute to the modulation of the emerging B cell repertoire. While in the perinatal period, positive selection of B cells predominates the adult repertoire is, in part, formed through negative selection. Examples of the action of such negative selection has been demonstrated in transgenic systems (*Russel* et al., 1991, *Hartley* et al., 1991, *Brombacher* et al., 1991). More recently, a striking example of negative selection of B cells utilising the V_H7183.1 gene segment in adult mice has been reported. This gene segment is utilised in >70 of the functional V_H7183 rearrangements during the perinatal period, while functional rearrangements of this gene is almost absent in adult peripheral organs (i.e. spleen, mesenteric lymphnodes, and Peyer's patches) (*Huetz* et al., 1992, *Decker* et al., 1991). Thus, during ontogeny a negative selection of B cells utilising the V_H7183.1 gene segments occurs. This negative selection of B cells appears to occur during the transition of B cells from the BM to the periphery since approximately 30% of the V_H7183.1-DJ_H rearrangements are productive in the pre-B and B cell compartment of the adult BM (*Huetz* et al., 1992).

IMMUNOPHYSIOLOGICAL REMARKS

Based on the data discussed above it could be hypothesised that the observed positive selection of perinatal B cells occurs on the basis of the properties of connectivity ascribed to these clones. Antigen receptor receptors with properties of "high connectivity" should be more likely to find complementary ligands in an immune system, which at this time is relatively "empty" in terms of antigen receptor specificities. In this context it is interesting to note that Kearney and co-workers who have found that hybridomas expressing the V_H7183.1 gene are among the most highly connected. These Ig molecules binds in ELISA assays other syngenic antibodies and antigens at a high frequency (*John Kearney*, personal communication). The binding to other sur-

face receptor Ig molecules at a high frequency may be a way to trigger other B cells to expand.

Since lymphocyte responses are a function of receptor occupancy the dose response curve is bell shaped, i.e. to few and to many receptors occupied by ligand leads to retention of the cell in the inactivated state (*Varela* and *Coutinho*, 1991), it might be argued that a highly connected antibody (e.g. V_H7183.1 encoded) would be positively selected as long as the B cell repertoire is expanding. Such clones would instead be negatively selected as the system "fills up", due to increasing receptor occupancy. The intrinsic bias of the rearranging machinery together with the minimisation of somatic diversification would guarantee the B cell repertoire to start as a highly connected idiotypic network, and thus give the system the means to establish, in a ordered and controlled fashion, the B cell repertoire.

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