The power of clonal selection

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ON page 271 of this issue[1] Lozano and colleagues unveil a new facet of the fascinating process by which cells expressing optimized antibodies are selected in the immune system. By doing so they document beautifully the evolutionary advantage of genetically equipping antibody-producing cells with a single antibody specificity. They also show that cells that have escaped this principle have the means to silence the expression of undesirable specificities later on.

As first postulated by Burnet[2], antibody-producing cells (B lymphocytes) are predetermined for the expression of antibodies of single specificity. At the genetic level, this is achieved by a principle which is abbreviated below as allelic exclusion: functional genes encoding the variable (V) regions of antibody heavy (H) and light (L) chains are generated through a process of gene rearrangements during B-cell development, and only one functional gene of each class is generated per cell[3]. The mechanism of allelic exclusion is still not fully understood, but there is strong evidence that it is controlled through the programme of gene rearrangements in a B-cell-autonomous fashion[4–7].

Why does the immune system make so much of an effort to produce monospecific B cells? One argument is that this is the most economical way of getting rid of autoaggressive cells through negative selection, while preserving the rest of the antibody repertoire. Another is that the clonal distribution of antibody specificities allows for the most efficient triggering of specific immune responses, through selective activation of only those cells that are selected for the appropriate antibody specificity. The force of these arguments was such that Burnet's theory of clonal selection quickly became the prevailing paradigm in immunology, long before its experimental demonstration.

Advantages

The evolutionary advantage of the clonal distribution of antibody specificities in the attempt to generate specific antibody responses could merely lie in the selective production of antigen-specific antibodies by terminally differentiated, antibody-secreting plasma cells. An additional possibility is that it also optimizes the expansion and selection of B cells in the response.

Lozano *et al.* now provide suggestive evidence for this latter mechanism, from an unexpected angle. In T-cell-dependent antibody responses, antigen-specific B cells undergo rapid and extensive clonal expansion in substructures of the immune system, the so-called germinal centres. During this process, somatic point mutations are introduced at high rate into the V-region genes of the proliferating cells. Only cells expressing high-affinity antibodies survive and become available as memory cells for secondary antibody responses. The amazing power of this process of positive selection implies that cellular survival depends on signals given to the cells in dependence of their receptor (that is, antibody) specificity (for review see ref. 8).

Lozano *et al.* used transgenic mice harbouring several copies of a rearranged L-chain gene encoding an L chain dominantly expressed in a particular T-cell-dependent anti-hapten response. The group had shown earlier that transgene-encoded L chains participate in this response, and that the transgenes are subject to somatic hypermutation as much as are endogenous V-region genes. They have now further analysed the structure and expression of the various transgene copies present in hybridomas isolated from the transgenic mice after they had been immunized and were secreting high-affinity antibodies carrying transgene-encoded L chains. Because the hypermutation process is random to a large extent within antibody V-region genes, one would expect that in a given cell only one of the multiple copies would have accumulated the particular point mutations leading to high-affinity hapten binding (as defined in earlier work[9]). Would the other copies of the transgene, mutated or unmutated, also be expressed by the cell?

Lozano *et al.* found most of the transgenes harboured by the four hybridomas analysed had undergone somatic mutation and were therefore distinguishable from each other at the messenger RNA level by nucleotide sequence. Furthermore, transgene-encoded L chains could often be distinguished by charge, as predicted from the variations in amino-acid sequence. For any given hybridoma, expression of the transgene turned out to be uneven at the levels of mRNA and/or protein. Translational termination codons had been introduced into some transgenes by the hypermutation process. This might have made the mRNA transcribed from those genes less stable and could have led to their relative underrepresentation in the cells. In other cases, transgenes appeared to be transcriptionally entirely silent, for unknown reasons. Most strikingly, in the two hybridomas in which one transgene copy had accumulated the two point mutations previously shown to confer to the antibody a ten times higher affinity for the hapten than that of the wild type, the expression of that particular transgene copy by far dominated that of the others.

Two biological messages are inherent in these results. First, antibody-producing cells, if artificially offered the opportunity to express multiple antibody specificities, have at their disposition a variety of mechanisms by which they can modulate the pattern of expression of the corresponding antibody genes. This is also suggested by the gradual counterselection of transgene-expressing cells at the expense of cells expressing endogenous immunoglobulin genes, as is generally seen in immunoglobulin-transgenic mice. Second, in the antibody response there is strong selection for monospecific cells, and cells homogenously expressing high-affinity antibodies win in the competition. Thus, although the mechanism of this selection process is not yet understood, the evolution of allelic exclusion is of obvious advantage for the organism in terms of the efficiency of the antibody response.

Stringency

More generally, the new results are yet another demonstration of the power of cellular selection operating in the generation of a functional B-cell compartment. The common principle in the various phases of B-cell development is the stringent selection against useless or non-optimal cells — be they cells that have not managed to express an antibody at the surface, that express high-affinity autoreactive antibodies, that fail to generate high-affinity antibodies when undergoing somatic hypermutation in antigen-driven responses, or (as Lozano *et al.* show in the present work and earlier data[6] had suggested) that have escaped allelic exclusion. (For review of these matters, see ref. 8.) In this sense, the mechanisms by which transgene expression is differentially modulated in the hybridomas of Lozano *et al.* may contribute to clonal selection as such. Moreover, working out these mechanisms may shed light on the problem of the quantitative control of gene expression beyond the cells of the immune system. □

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