Effects of age on antibody affinity maturation

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Abstract

The elderly are more susceptible to infectious diseases. Mortality and morbidity from infections increase sharply over the age of 65 years. At the same time, the efficacy of vaccinations in the elderly is decreased. The elderly also have an increased incidence of cancer and inflammatory diseases. All the above indicate an age-related dysregulation of the immune system. Evidence suggests that the change in the humoral immune response with age is a qualitative rather than a quantitative one, i.e. it is the affinity and specificity of the antibody that changes, rather than the quantity of antibody produced. There are a number of possible causes of this failure, one of which is a defect in the mechanism of hypermutation of immunoglobulin genes. We have studied individual clonal responses within germinal centres of spleen and Peyer's patches in young and old patient groups. Our results indicate that there is no difference in the actual mechanism of hypermutation with age. There are, however, differences that are due either to a change in selection processes or to a change in the founder cells available for activation.

Infection is one of the leading causes of mortality in people over the age of 65 years. It is also an important cause of morbidity – hospitalization due to infection is much more common in the elderly. The mucosal immune system is the first line of defence against pathogens encountered after ingestion and inhalation, and the decline of mucosal immune responses with age is particularly significant [1]. Mortality from gastrointestinal tract infections is increased 400-fold in the elderly, and mortality from pneumonia and influenza is increased 120-fold. The mucosal and systemic immune systems appear to be separate, with cells activated in a mucosal site homing back to that site or to another site within the common mucosal immune system [2]. Thus, when studying the problems of immune senescence with age, it is important to consider the different tissues and routes of infection.

Vaccinations are an invaluable tool in combating infection. However, the decline in effectiveness of the immune system with age means that the currently available vaccines and vaccine regimes are less effective in the elderly. It has been shown that the antibody response in older people is composed of antibodies with a lower affinity for the immunizing antigen compared with those produced in a younger person. In addition, the number of autoantibodies produced during an immune response is greatly increased in the elderly [3]. In fact, the overall level of autoantibodies in the elderly person is generally high, even though there may not be the associated pathology of autoimmune disease. Thus, although the quantity of antibodies produced in old age does not change, the quality of the response differs.

High-affinity antibodies are produced as a consequence of affinity maturation, a process occurring in the germinal centre of B cell follicles. When a B cell is activated by antigen, it enters the germinal centre, proliferates, and mutations are accumulated in the Ig gene. The newly encoded antibody is expressed on the surface of the B cell and a process of negative selection occurs whereby, unless the B cell receives rescue signals based on the affinity of the new antibody for its antigen, it undergoes apoptosis. If the B cell receives the appropriate rescue signals, it survives and then goes on for further rounds of mutation and selection, develops into memory B cells against future antigen challenge, or develops into an antibody-secreting plasma cell to combat the infection immediately [4]. Thus the following question arises: if the antibodies produced in old age are of lower affinity, at what point is the affinity maturation process impaired? We have addressed the question of whether the hypermutation mechanism itself is at fault, or whether there is a problem with the process of selection.

Reports about whether hypermutation changes with age are conflicting. Two reports on hypermutation in humans have shown either an increase in hypermutation with age, or no change [5,6]. The problem with these studies is that they look at the number of mutations per Ig gene at a particular time point, and this does not distinguish between disparities due to a difference in the mechanism of hypermutation and those due to a difference in the activation history of the individual, or in the tissue of origin.

We have circumvented these problems by looking at individual clonal responses in the germinal centres, both of the spleen and of the Peyer's patches in the gut. This was accomplished by microdissecting areas of germinal centre B cells from immunohistochemically stained cryosections, and using PCR to amplify the Ig genes. Ig genes from clonally related B cells can be identified by virtue of their complementarity-determining region 3 (CDR3). Clones with related Ig genes can be collated, and a family tree can be

Key words: affinity maturation, germinal centre, hypermutation, immunoglobulin, lineage tree.
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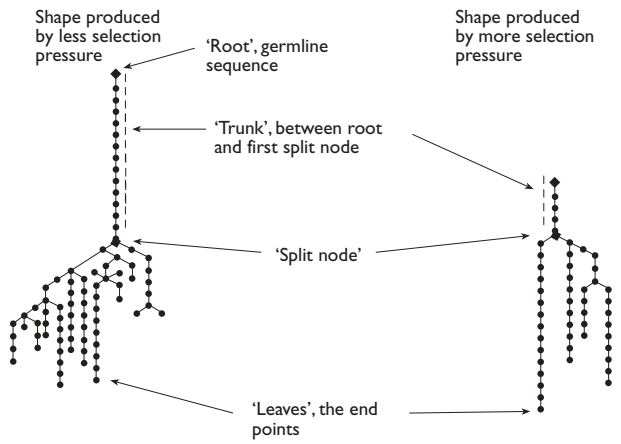
Figure 1 | Examples of mutational lineage trees isolated from germinal centre B cells

The examples illustrates the difference between a tree that indicates that a high degree of selection has taken place and one that indicates that a lesser degree of selection has taken place. The nomenclature of features in lineage trees is shown.



composed by analysis of the mutations acquired during the germinal centre reaction (Figure 1). Tree shape parameters can then be quantified further by an algorithm that we have recently developed [7]. Since the process of affinity maturation proceeds by the addition of one, or possibly two, mutations in between successive rounds of selection, the rate of hypermutation for different B cell clones within a germinal centre can be compared by looking at the average path length in the lineage trees produced. Measuring hypermutation in this way indicates that there is no difference in the level of hypermutation occurring during the germinal centre reaction with age [8]. Interestingly, this measurement also shows that there is no difference in the rate of hypermutation between genes isolated from the Peyer's patch and those isolated from the spleen [8]. This is evident in spite of the fact that Ig genes from the Peyer's patch and lamina propria of the gut have consistently shown a higher total level of mutation in other studies [9]. This lends credence to the idea that observed levels of hypermutation can reflect repeated activation of B cells, i.e. the founder B cells in reactions of the Peyer's patch have already been mutated previously.

The shapes of the same lineage trees were studied in order to determine whether there are any differences in the selection of B cells in the germinal centre response with age. A high level of selection, so that very few daughter cells from each different round of proliferation and mutation survive to re-enter the process, would be reflected by a lineage tree with long 'branches' and few split nodes. Conversely, a low level of selection would result in more daughter cells surviving, and the lineage tree would assume a more bushy shape, with shorter branches and a higher number of daughters per split node (Figure 1). Our lineage trees show that selection of B cells in the germinal centre increases with age in the spleen, yet decreases with age in the Peyer's patch [8]. An alternative method of determining selection of Ig genes uses the ratio of replacement to silent mutations in the different areas of the Ig gene responsible for antigen binding compared with antibody framework structure. This allows the inclusion of all Ig genes found in germinal centres, rather than just those that can be allocated to a lineage tree, and so uses a wider range of samples. The results of this analysis are the same: selection appears to increase in the splenic germinal centre, yet decreases in the Peyer's patch germinal centre, with age [8]. It would therefore appear that a decrease in antibody affinity is not due to perturbations in hypermutation, but that there are significant differences in the way that the antibodies are selected in old age.

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Received 11 November 2002