REGULATION OF MEMORY B CELL DIFFERENTIATION

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Lymphocyte precursors originating in the bone marrow undergo several antigen independent differentiation steps to give rise to a population of direct antibody forming cell (AFC) progenitors. These cells are committed to eventual function and antigen reactivity and on antigenic stimulus differentiate to high rate antibody secreting cells and memory cells. Differentiation is associated with drastic clonal expansion and appears to be regulated by T cells that govern the overall level of the humoral response. Taken together, the regulatory cells and cells along the developmental pathway from precursor to effector for a given response, constitute one or in most instances a series of networks.\(^1\)

Interactions between T and B cells, be they based on idiotype\(^2\) or allotype\(^6\) recognition are reflected in the behavior of B cell populations either as changes in antibody secretion\(^2-5\) or memory cell generation.\(^7\) Mice that contain T suppressor cells (Ts) that regulate Ig-\(\lambda\) (Ig\(_{\lambda}\)) synthesis and secretion (Ig-\(\lambda\) Ts) do not express serum Ig-\(\lambda\) nor do they produce specific Ig-\(\lambda\) antibodies when challenged with antigen\(^6\); however, these same animals do generate populations of low avidity Ig-\(\lambda\) memory B cells. This memory population cannot mature in avidity in suppressed mice and can only be revealed when Ig-\(\lambda\) Ts are eliminated.\(^7\) Hence, although the mechanism of blockade is unknown, it can be concluded that suppressive regulation of the Ig-\(\lambda\) network results in an early arrest of antigen driven B cell differentiation.

Ts regulation of Ig-\(\lambda\) antibody formation is complex and indirect. The suppressor cell secretes a factor that removes (kills or inactivates) an Ig-\(\lambda\) specific T helper cell (Ig-\(\lambda\) Th)\(^8\) while having no apparent effect on precursors of Ig-\(\lambda\) AFC.\(^7\) These observations suggest that a number of different T cells direct B cell development and fine analysis of B cell differentiation would greatly increase the resolution of events occurring during Ig-\(\lambda\) responses.

We have used two approaches to conduct this analysis. Firstly, we have examined the expression of Ig\(_D\) by memory B cells that arise at different times after priming. These experiments stem from earlier studies that demonstrate that the memory B cell pool is heterogeneous and can be subdivided on the basis of surface Ig\(_D\) expression.\(^9\) As a second level of analysis we have measured the
avidity distribution of antibodies secreted by the progeny of these memory cells. Taken together these methods enable one to separate the virgin B cell-memory B cell-afc route into several components and reveal the cellular interactions and selective pressures that operate on each component.

In these experiments IgD⁺ and IgD⁻ memory B cells were isolated from primed whole splenic B cells using fluorescein conjugated monoclonal anti-Ig-5b serum 11 (IgD of the b allotype) and the fluorescence-activated cell sorter (FACS). 2. The immune potential of these memory populations was revealed on adoptive transfer with carrier-primed T cells and antigen. Avidity analyses were performed either directly on afc arising after transfer or by free hapten inhibition of serum antibody binding to target in a solid-phase radioimmune assay (RIA).

Our findings are summarized in Fig. 1, which describes the differentiation pathway of Ig-1b precursors to Ig-1b afc. This figure presents the pathway as linear although there are points where separate branches could be considered. The B cells along the path are virgin cells, memory cells with surface IgD (IgD⁻), memory cells lacking surface IgD (IgD⁺) and afc.

Fig. 1. Regulation of Ig-1b Memory Cell Differentiation
C.T.H. = carrier-specific helper; Ig-1b Th = Ig-1b helper cell; Ig-1b Ts = Ig-1b suppressor cell.
Virgin B cells capable of generating T dependent adoptive primary responses are IgD+9. Since few or no IgD+ IgM+ B cells are present in unprimed mice (assessed by an anti-Ig-5b serum) we presume the virgin B cell also expresses surface IgM.

Antigen induces virgin B cells to give rise to afc and memory cells. If the antigenic stimulus is weak predominantly IgD+ memory is generated. These memory cells transfer a response of similar avidity to that of unprimed B cells. Antibodies secreted by progeny of IgD+ memory cells are of low avidity and special procedures must be followed to effectively assay them. In practice we study anti-trinitro phenyl (TNP) responses in RIA using TNP bovine serum albumin (TNP-BSA) as target antigen. We find that most antibodies produced by IgD+ anti-TNP memory cells are detectable on conjugates of TNP-BSA but not on TNP-BSA. IgD+ Ig-lb memory cells are generated in Ig-lb suppressed mice. Thus the virgin B cell to IgD+ memory cell route can be considered to be independent of help from Ig-lb Th.

If a strong antigenic stimulus is supplied during priming, IgD+ memory cells are generated and these are rapidly replaced by IgD+ memory cells, which produce high avidity responses on adoptive transfer.9 The high avidity response from progeny of IgD+ memory cells is fully revealed on TNP-BSA in RIA. The rate at which IgD+ memory B cells are generated varies between different mouse strains and can be accelerated by boosting or including killed B. pertussis organisms with the priming antigen. Ig-lb suppressed mice maintain Ig-lb IgD+ memory cells under conditions where all other memory cells in normal or suppressed mice become IgD-. A few Ig-lb IgD+ memory cells do, however, arise in suppressed animals and this number is related to how suppressed the cell donor is at the time of priming. We conclude from these studies that IgD+ to IgD+ Ig-lb memory cell differentiation requires Ig-lb Th.

In a series of double transfer or parking experiments using FACS-isolated IgD+ and IgD+ memory cells we have shown that IgD+ memory cells directly give rise to IgD+ memory cells. This step is associated with clonal expansion. IgD+ memory cells are self renewing. The IgD+ to IgD+ and the IgD+ to IgD+ differentiation paths need to be antigen driven and depend on the presence of a carrier-primed T cell (J. Black et al., manuscript in preparation).

We are aware that these data conflict with results obtained by Drs. Zan-bar and Strober, which are also reported in this book. At present this conflict is not resolved but a possible explanation can be advanced to explain the differences in our analyses. The priming regime followed by Zan-bar and Strober would be expected to generate predominantly IgD+ low avidity memory whereas the assay
used to detect ac progeny of these and IgD memory cells after transfer probably detects antibody of intermediate to high avidity more efficiently than antibodies of low avidity. Since Strober and Zan-Bar find identical responses from IgD and IgD memory cells in their first host it is likely that many more IgD than IgD memory cells were transferred into these animals. This initial imbalance in memory cells packed in the first host would be revealed on second transfer.

Other explanations for the differences between our studies hinge on the possibility that the hetero-anti-IgD serum used by Strober and Zan-Bar reacts with a cell surface determinant other than IgD. At present there is no evidence to support this possibility.

Our studies show that avidity maturation of Ig-1b immune responses is orchestrated by a variety of T cells (Ig-1b Th, carrier Th and Ig-1b Ts), which in conjunction with antigen control IgD to IgD differentiation and clonal expansion. Mice that are suppressed for Ig-1b production contain Ig-1b Ts that inactivate Ig-1b Th and thus prevent IgD to IgD differentiation of Ig-1b memory B cells. Ig-1b memory cells in a suppressed mouse are thus blocked at the IgD stage and high avidity clones cannot be selectively expanded from the pool during differentiation to the IgD state. We presume that high avidity memory cell expansion is due to or accompanied by antigen selection. Ig-1b regulation resembles idioype regulation in many ways and the above differentiation process involving carrier-specific helper T cells and Ig specific helper T cells may be common to B cells of all lineages.

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