Lymphocyte differentiation

Control of antibody production

from Miranda Robertson

THE differentiation of B lymphocytes is of particular interest from two points of view. To the molecular biologist the singular behaviour of the immunoglobulin genes, which undergo rearrangements that profoundly affect their expression, offers an opportunity to study the basis of eukaryotic gene regulation. From a more practical point of view, the cellular interactions that later control the proliferation and maturation of immunoglobulin-bearing B lymphocytes are crucial to the regulation of immune responses. The problem of gene expression has been approached with cloned DNA; that of immune regulation with cloned cell lines. Some progress on both fronts was reported at a recent meeting in Basel^{*}; and in the latest issue of the *Proceedings of the National Academy of Sciences U.S.A.*¹, Rice and Baltimore report the forging of the first link between cellular and molecular regulation of antibody production.

The gene rearrangements that take place in lymphocytes of the B lineage bring the DNA encoding the variable (V) region of the immunoglobulin molecule, which is transcriptionally silent, into proximity with the DNA encoding the constant (C) region, which is constitutively transcribed^{2,3}. After rearrangement, the complete gene, comprising both V and C segments, is transcribed at a significantly higher rate. It has been suggested² that this amplification may be the combined effect of the upstream promoter of the V segment and the structure of the chromatin in the region of the C segment. Further amplification of the rate of transcription can then be induced by external signals.

In an attempt to elucidate the mechanisms underlying these amplifications, people in several laboratories have been transfecting cells of both lymphoid and non-lymphoid lineages with cloned immunoglobulin genes. Earlier this year, Falkner and Zachau⁴ reported that cloned κ light-chain genes in non-lymphoid cells were not transcribed under the influence of their own promoters, although transcription could be demonstrated if they were placed under the control of a viral promoter. They suggested two possible explanations for this failure — either immunoglobulin gene expression may depend on tissue-specific factors not synthesized in non-lymphoid cells; or alternatively, transcription may depend on chromatin structure in ways that could be influenced by sequences upstream or downstream of the cloned DNA.

In the light of results reported by Richard Maki (Massachusetts Institute of

Technology), the former possibility can be ruled out, at least for heavy-chain expression. Steven Gillies, working in the laboratory of Susumu Tonegawa (Massachusetts Institute of Technology), has used both λ and κ light-chain clones and a $\gamma 2b$ heavy-chain clone to transfect a mouse fibroblast line and finds that the heavy chain is transcribed under the control of its own promoter. The λ and κ transcripts, however, appear for unknown reasons to be longer than those of the myeloma cells in which the genes originated.

Meanwhile, evidence of a different kind increasingly implicates chromatin structure in the regulation of immunoglobulin gene transcription. For example, Robert Perry and his colleague Elizabeth Mather (Institute for Cancer Research, Philadelphia) have shown that the state of methylation of immunoglobulin genes is determined by their chromosomal location. It is a general (though not invariable) rule that DNA sequences are undermethylated in those cells in which they are transcribed. Perry and Mather find, in conformity to this general rule, that the rearranged V-gene segments in plasmacytomas are undermethylated. But unrearranged V genes in the same nucleus, and with virtually identical sequences, are methylated. They accordingly suggest that the formation of a transcriptionally active chromosome domain may depend on the overall DNA context of the region, the lack of methylation serving to stabilize rather than to establish the necessary structure.

The end result of successful rearrangement of the immunoglobulin genes is the expression of an immunoglobulin M molecule on the surface of the B cell; and here begin the problems of cellular immunologists seeking to discover how the humoral immune response is controlled. The nature of the early signals that drive the small resting B cell to the blast stage, at which it becomes susceptible to the signals inducing proliferation and immunoglobulin secretion, is controversial; but the early stimuli can be mimicked *in vitro* by bacterial lipopoly-saccharide (LPS), one of whose effects is to increase still further the rate of transcription of surface immunoglobulin.

Rice and Baltimore¹ have now succeeded in demonstrating LPS inducibility in a cloned light-chain gene introduced into cells of a lymphoid line, and have thus opened an approach to the question of how such signals influence gene expression. At this stage, they are able to conclude only that the sequences necessary for LPS inducibility must lie within 1.5 kb on either side of the coding sequence, and they do not know whether induction is direct or

whether the direct effect is on heavy-chain transcription which in turn regulates light-chain transcription.

In the exploration of the later signals that induce the final maturation of B lymphocytes, attention has focussed increasingly on those B-cell responses that depend on T-cell help, because the development of cloned T-cell lines promises to clarify at last the cellular interactions underlying them^{5,6}.

The isolation of later-acting factors produced by T-cells is now well under way; but if this is making T-B cell interactions clearer, it is certainly not making them simpler. It is now clear, for example, that distinct factors are necessary to induce proliferation of B-cell blasts and to stimulate their maturation into antibody-secreting plasma cells. Factors stimulating proliferation have been isolated from T-cell lines or T hybridomas by Howard *et al.*⁷, Lernhardt *et al.*⁸ and P. Petterson (Sweden). The factor isolated by Lernhardt *et al.* is known as BRF and is assumed to represent the replication-inducing activity in B-cell replication and maturation factor (BRMF), which was originally extracted from uncloned T cells and induces both replication and antibody secretion⁹.

The attempt by Maureen Howard and her colleagues (NIH) to use a similar (identical?) factor, B-cell growth factor (BCGF), to maintain long-term clones of immature B cells has revealed further unsuspected complexities in B-cell differentiation. She can induce proliferation in B-cell blasts with a combination of BCGF and interleukin-1, which is produced by macrophages; but not with either alone. BCGF is apparently necessary to drive the cell through the early phases of the mitotic cycle, and interleukin-1 for later phases. Even with both these factors, however, the growth of B cells is sluggish and the expectation that they could be used to maintain long-term clones¹⁰ has been disappointed. Hopes are now pinned on two more recently isolated factors: B15-TRF, which may promote later rounds of cell division; and EL-TRF, which resembles biochemically the T-cell growth factor interleukin-2 but acts on B cells rather than T cells. □

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^{*}A symposium on 'B cell differentiation' was held at the Basel Institute for Immunology on 13–15 October 1982.

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