and terminating past ε is produced in $\mu^+ \varepsilon^+$ lymphocytes. This approximately 180 kilobase multi-C_H transcript could yield μ and ε membrane mRNAs by differential RNA splicing coupled with specific poly(A) addition (see the figure). Similar studies on a population of $\mu^+ \gamma 1^+$ B cells from normal mouse spleen have been interpreted in the same way[11]. However, cellular immunologists will want clear assurance that the immunoglobulin molecules on the surface of these cells are actually synthesized by them and not picked up from other cells, especially since the existence of a multi-C_H transcript in immature B lymphocytes may be very difficult to establish (none has yet been isolated for the μ–δ transcription unit). This hypothesis poses the intriguing problem of a mechanism for operating the switch first by RNA splicing and then by DNA rearrangement since γ^+ and α^+ B cells have been shown to give rise to plasma cells which produce the same heavy chain isotype.

Another hypothesis arises from observations on subclones of a pre-B cell line (18–81) transformed by Abelson virus. In these cells, switching from μ to γ2b (ref. 12) can apparently occur in the absence of μ-gene deletion, though not without some DNA rearrangement[13]. It is possible that at some point in the history of the 18–81 cell line, the δ, γ3 and γ1 genes were deleted and the γ2b gene was positioned immediately 3′ to the μ gene where it could give rise to a μ–γ2b transcript by the same mechanism that produces the μ–δ transcript. F. Blattner (University of Wisconsin) suggested that this might be a transitional step in isotype switching, the constant-region gene to be expressed in each case replacing the δ gene 3′ to μ. The next step would involve C_μ gene deletion before plasma cell differentiation.

Successive and direct switching

So far, there is evidence for both successive and direct isotype switching (see the figure). Both switching modes could operate on a purely stochastic basis[5], or under the influence of regulatory elements such as isotype-specific recombinases[14].

In addition to direct μ→γ and μ→α switches, M. Wabl (University of Tübingen) reported evidence for a μ→ε switch in lipopolysaccharide (LPS)-induced plasma blasts. Successive isotype switches are also suggested from studies using the splenic focus assay and limiting dilution as methods for examining these cell clones[15,16]. Using the limiting dilution of LPS-stimulated cells, A. Coutinho and co-workers (Umeå University) obtained results consistent with switches from μ to each of the other isotypes and for multiple patterns of successive 'downstream' switches. In the response to trinitrophenol-Ficoll, a T-independent antigen, the frequency of γ isotypes expressed follows the C_H gene order (that is, γ3>γ1>γ2b>γ2a), and results of splenic focus analysis suggested that γ3 cells may be pivotal for switches to other γ isotypes[16]. K. Marcu (SUNY, Stony Brook) showed that $S_{\gamma 3}$, S_α and S_ε possess extensive homology with S_μ, while $S_{\gamma 1}$, $S_{\gamma 2b}$ and $S_{\gamma 2a}$ have very limited S_μ homology, and suggested that this could provide a genetic basis for intermediate $S_{\gamma 3}$ switches to other γ isotypes (see the figure). Marcu also presented evidence for a new consensus DNA sequence, YAGGTTG, preferentially located 5′ of C_H switch sites and repeated in S regions[17,18], which, together with previously identified common S sequences (GAGCT and GGGGT)[19], may play a part in switch-site recognition[17–19].

Successive C_H gene switches have recently been explored at the molecular level in C_H switch-variant cell lines[20,21]. A switch from γ2b to γ2a in MPC-11 myeloma variants results in S region-mediated γ2b gene deletion[20]. F. Sablitzky (Genetics Institute, University of Cologne) presented some startling results from correlations of C_H gene rearrangements and deletions with isotype switching in clones of spontaneous class-switch variants derived from the X-63 hybridoma[15]. In X-63 hybridoma cells, 'backswitches' (for example γ2a→γ1) occur at 100-fold higher frequency than forward switches (for example γ1→γ2b→γ2a), which arise at frequencies of 10^{-7}. The unprecedented finding of C_H backswitching could be most simply explained by either trans-homologous chromosome exchange or the presence of duplicated γ genes generated by unequal cross-over during sister chromatid exchange. Even though these findings may partly reflect peculiarities of the cell lines, they do clearly demonstrate that successive switching — for example, γ1→γ2b→γ2a — can occur.

Role of T cells in isotype expression

It is well known that the nature of the antigen and responding T cells or their helper and suppressor factors can selectively influence the isotypes expressed by plasma cell progeny of responding B cells. J. Davies (Washington University, St Louis) reviewed evidence indicating that T-independent polysaccharide antigens elicit primarily μ and γ3 antibodies while the T-dependent protein antigens preferentially induce μ, γ1 and γ2 antibodies in the mouse. Coutinho showed that the same hapten can elicit different antibody isotypes when coupled to different carriers which determine the nature of the T-cell help. Y. Rosenberg (MRC, Mill Hill) showed that the isotype-specific influence of antigen-stimulated T cells may spill over to cause bystander B-cell clones to undergo plasma cell differentiation, the T-cell influence presumably being mediated by soluble factors.

Three groups of investigators represented at the Umeå meeting have analysed the immunoglobulin isotypes expressed by mouse B cells when cultured with established T-cell lines (A. Coutinho; and L. Forni, Basel) or soluble T-cell factors (P. Tucker, Dallas; & E. Severinsson, University of Stockholm). In each system, the polyclonal B-cell activator LPS by itself induced γ3 more than γ1 plasma cells. The addition of activated T cells, or soluble factors released from them favoured the reverse pattern, γ1 over γ3. L. Forni's data suggested that LPS induces both γ1 and γ3 B-cell blasts to proliferate, but only the γ3 blasts to differentiate into mature plasma cells; the subsequent addition of activated T cells, by contrast, reduces the frequency of γ3 cells and induces γ1 plasma cells. P. Tucker presented data on the state of the γ3 and γ1 mRNA species before and after the addition of a T-cell product (B-cell differentiation factor[22]) to LPS-stimulated B cells. LPS alone induced γ3 mRNAs, but very little γ1 mRNA. Following the addition of the T-cell factor, γ1 mRNAs were increased in quantity and γ3 mRNAs reduced. But all these results, while tantalizing, leave open the central question: do T cells instruct B cells to switch isotypes or do they select isotype-committed B cells for preferential induction of terminal differentiation?

Clearly, we still have important lessons to learn about the genetic basis for C_H isotype switching and its regulation. While solutions appear almost within reach, these genetic events remain elusive as they occur transiently and in minor subpopulations of cells. Clones of normal B cells undergoing isotype switches are unavailable. The C_H switching phenomenon may continue to challenge both cellular and molecular biologists for some time □

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Corrigendum

In "Sons and daughters" from T.H. Clutton-Brock (*Nature* **298**, 11; 1982) the figures shown for *Papio cynocephalus* (Table 2) as "Per cent males" are actually totals; the percentage of males would be 35 (for high maternal rank) and 68 (for low maternal rank).