The basic difficulty, however, is straightforward. As Churchland[4] first pointed out, Libet's argument for "an automatic subjective referral of the conscious experience backwards in time"[3] depends crucially on his assumption that the sensation following a train of (individually subthreshold) shocks to the cortex starts immediately, or almost immediately, after the shock that makes the train long enough to be effective; in other words, that there is only a very short latent period. The arguments that Libet used[5] in reply to Churchland's criticisms, arguments which attempted to show that the latent period must be extremely short, are themselves unsatisfactory[4]. Because we do not know the duration of the latent period, any assumption about its length must be arbitrary, though not all assumptions are equally plausible. Libet is correct in pointing out that if the latent period is indeed very short, his results appear to lead to a surprising and exciting, though not impossible, conclusion. But until the assumption is supported by evidence, scepticism is more advisable — if less enjoyable — than excitement.

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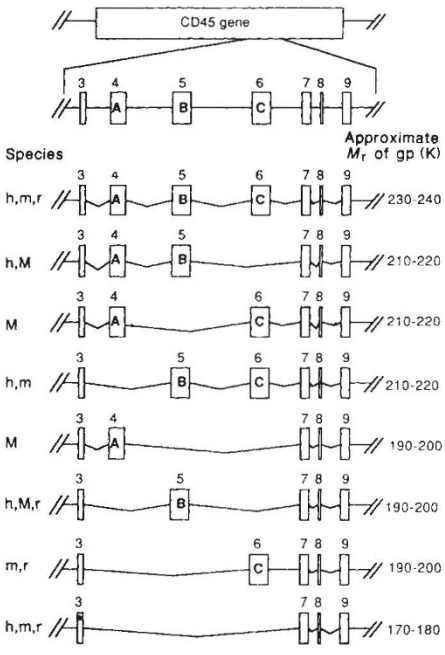
Confusion over CD45 isoform

SIR — Bell and Sparshott report [1] the apparent interconversion of rat T-lymphocyte populations expressing distinct isoforms of CD45, the leukocyte common antigen. Their extrapolations of the data presented are disturbing, however.

CD45 isoforms are generated from a single gene by the alternative use of three variable exons termed A, B and C (corresponding to exons 4 to 6 of the CD45 gene, see figure)[2–5], and isoform-specific CD45 antibodies are classified according to the exon on which their reactivity depends. For example, CD45RA reagents bind epitopes encoded by exon A on several high molecular mass CD45 isoforms, and CD45R0 indicates specificity for the smallest isoform, which lacks sequences encoded by exons A, B and C (ref. 6).

In humans, CD45RA and CD45R0 define largely reciprocal T-cell populations[7]. When activated *in vitro*, CD45RA T cells acquire CD45R0 (ref. 7). Frequencies of T-cell memory responses are high among CD45R0, but low among CD45RA populations, suggesting that the stable acquisition of



Schematic representation of the CD45 gene (based on refs 2–5, 12, 14). A, B and C, variable exons (4 to 6) as assigned by Streuli *et al.*[5]; h, m and r, human, mouse and rat cloned cDNAs, respectively; M, murine isoforms demonstrated by polymerase chain reaction[11]; numbers on the right, relative molecular mass (M_r) for translated and glycosylated products[14].

CD45R0 determinants may accompany human T-cell priming *in vivo* as well as *in vitro*[8–10]. Bell and Sparshott propose a revision of this hypothesis[1]. In their study, rat T cells are enriched or depleted for CD45RB expression with the CD45RB reagent Ox22 (ref. 11), injected into nude (T-cell deficient) recipients, and allowed to expand for several weeks or months. CD45RB-enriched, as well as CD45RB-depleted, populations give rise to progeny of mixed CD45RB positive/negative phenotype. This finding, the authors conclude, is incompatible with the stable aquisition of CD45R0 during human T-cell memory formation[7]. The apparent conflict hinges on the assumption that CD45RB negative ($Ox22^-$) T cells necessarily express CD45R0 (ref. 1). A review of the possible CD45 isoforms lacking exon B-encoded sequences (see figure) shows that this equation is not justified: three variable exons allow eight permutations, at least six of which have been isolated as complementary cDNA clones[2–5]. Confirmation of all eight isoforms comes from studies using the polymerase chain reaction with exon-specific primers[12]. At least one cloned rat CD45 cDNA lacks exon B but contains exon C (ref. 3), and immunoprecipitation analysis indeed indicates that not all high molecular mass rat CD45 species are recognized by Ox22 (ref. 11). CD45RB-positive as well as CD45RB-negative rat T-cell populations are therefore heterogeneous in terms of their CD45 isoform expression, and conclusions regarding the gain or loss of CD45R0 cannot be drawn from the experiments reported by Bell and Sparshott[1].

Extrapolation of the data is also tenuous because, in humans, unlike rats, CD45RB is expressed by the great majority of T lymphocytes[13] and (in contrast to CD45RA and CD45R0) has not been established as a market for T-cell heterogeneity.

The generation of antibodies specific for rat CD45RA and CD45R0 might clarify the situation. Alternatively, methods such as the polymerase chain reaction could be employed to characterize CD45 isoform expression by rat T-cell populations. But more general obstacles complicate the analysis of progeny derived from large, heterogeneous precursor populations. To rule out the possibility that contaminating cells are responsible for their results, Bell and Sparshott inject mixtures of allotype-marked T cells, enriched or depleted for CD45RB expression. They find CD45RB-positive and negative progeny of both allotypes, and conclude that all injected cells expand equally well. The data would also (and perhaps more in line with other haematopoietic reconstitution models) be explained by the presence of specific precursors giving rise to heterogeneous progeny of both allotypes. Donor-derived cells undergo extensive initial proliferation, but T-cell numbers reach relatively stable levels some time (as judged by the relative expansion reported after 3 weeks and 7.5 months). The chimaeras are therefore able to regulate their peripheral T-cell pool, and homeostatic mechanisms could also explain stable ratios between CD45RB-positive/negative T lymphocytes.

If these problems can be resolved, the approach taken by Bell and Sparshott may bring a constructive revision of current concepts regarding CD45 isoform expression during T-cell ontogeny. As it stands, such conclusions seem premature.

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