Immunology

Investigating T-cell memory

Memory is a long-recognized, crucial and poorly understood property of adaptive immunity. Jacob and Baltimore[1] have designed an elegant genetic approach to marking memory T cells and their precursors irreversibly and have obtained intriguing results[1,2]. I suggest that the particular enzyme (human placental alkaline phosphatase) chosen for irreversible marking in postnatal life contributes to certain features of this system and perturbs the homeostatic mechanisms of T-cell activation and death.

Jacob and Baltimore used Cre-recombinase-mediated genetic recombination, driven by the granzyme-B promoter, to obtain irreversible and selective surface expression of placental alkaline phosphatase (PLAP; driven by the CD2 promoter) in T cells activated by antigen in adult life. In principle, this system should result in the irreversible marking of activated T cells and enhance the visualization, purification and fate mapping of activated T cells and memory T cells. As such, it should represent an invaluable tool for analysing T-cell memory, a subject central to the pathophysiology of immunity and vaccine development[2].

However, this genetic marking approach yielded some unexpected and unexplained results[1,2]. For instance, in unimmunized mice, none of the T cells expressing high levels of CD44, which have presumably responded to environmental antigens, was positive for the PLAP marker; upon exposure to antigen, only a fraction of activated T cells became PLAP-positive. During a response to lymphocytic choriomeningitis virus (LCMV), only about 10% of LCMV-specific T cells became PLAP-positive.

Different forms of alkaline phosphatase have been described[3]. Human PLAP shows only limited homology to the mouse isoforms, sharing 55% identity with mouse PLAP for example[4]. Antibodies against human PLAP have thus been generated easily in various animal species, including mice[3], so expression of human PLAP in T cells activated in adult life may result in a cellular and/or humoral anti-human-PLAP immune response.

An anti-human-PLAP response could explain some of the unusual features of the results obtained from irreversibly marking activated T cells with this human enzyme. For instance, selective pressure may favour weakly expressing T-cell clones. In general, the activation of immune responses directed against activated T cells may perturb the system in an unphysiological way.

These considerations caution against the overinterpretation of results derived from the indelible marking of activated T cells with human PLAP. To be informative, the same genetic approach should take advantage of non-immunogenic tracers.

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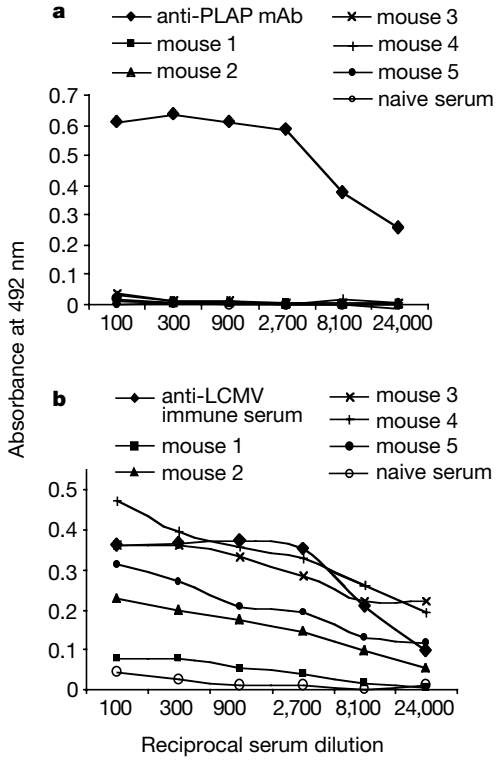

Figure 1 Antibody production by doubly transgenic mice infected with lymphocytic choriomeningitis virus (LCMV). **a,b,** Mice do not produce antibodies against placental alkaline phosphatase (PLAP) (**a**), but are able to mount a good antibody response against LCMV (**b**). For enzyme-linked immunosorbent assays, 96-well flat-bottom Immunoplates (Nunc) were coated with purified PLAP protein (Anawa Trading SA, Zurich; 80 ng per well) for 2 h at 37 °C (**a**) or with a lysate from LCMV-infected BHK-21 cells (**b**). Plates were blocked with 4% non-fat skimmed milk and 0.1% Tween 20, and duplicate dilutions of serum from GranzymeB–Cre × CD2–STOP–PLAP doubly transgenic mice (infected 9 d previously with LCMV Armstrong) were allowed to bind for 90 min. A monoclonal antibody against human PLAP (clone 8B6; Sigma) and LCMV immune serum were used as positive controls for detecting PLAP and LCMV, respectively. After extensive washing, plates were incubated with goat anti-mouse IgG conjugated to horseradish peroxidase (Sigma) for 90 min. After washing, the substrate *o*-phenylenediamine (Sigma) was added and the colour developed was quantified 30–40 min later on a Spectramax 340 plate reader (Molecular Devices) at 492 nm (ref. 3). Background absorbance from wells with no antigen was subtracted from antigen-containing wells at each dilution. The absorbance at 492 nm is plotted against the reciprocal of the serum dilution.

Maris et al. *reply* — Mantovani suggests that expression of human placental alkaline phosphatase in activated T cells might stimulate an immune response against PLAP and that the anti-PLAP antibodies generated could lead to the elimination of $CD8^+$ T cells expressing large amounts of PLAP on their surface. We therefore tested whether doubly transgenic (GranzymeB–Cre × CD2–STOP–PLAP) mice could mount an antibody response against PLAP during the course of a response against LCMV, and whether the serum of these mice contained antibodies against human PLAP.

We infected doubly transgenic mice ($n = 15$) with 5×10^4 plaque-forming units of the Armstrong strain of LCMV and nine days later collected their serum to test for the presence of antibodies against human PLAP in an enzyme-linked immunosorbent assay (ELISA). None of the fifteen LCMV-infected doubly transgenic mice produced anti-PLAP antibodies, as shown by a representative ELISA from five LCMV-infected (day 9 post-infection) doubly transgenic mice (Fig. 1a).

To find out whether these mice mount a strong antibody response against LCMV, we tested the serum from the same 15 mice for antibodies against LCMV and found that 14 of the 15 mice mounted strong antibody responses against this virus. Figure 1b shows the anti-LCMV antibody levels in the same five mice that were used to obtain the results shown in Fig. 1a — four out of five mice mounted strong anti-LCMV antibody responses.

These results indicate that the low frequency of PLAP-positive $CD8^+$ T cells (10%)[1] compared with the LCMV peptide/MHC tetramer-positive $CD8^+$ T cells (70%)[2] observed in the acute phase of the LCMV response is not due to an antibody response against the human PLAP reporter and antibody-mediated elimination of PLAP-expressing $CD8^+$ T cells. But we still need to investigate why PLAP marks only a subset of the activated $CD8^+$ T cells.

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