Accelerated development of a Th-2 type factor in animals with and without an imbalance between help and suppression

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Summary. A helper factor can be detected in antigen-treated supernatants from spleen T and adherent cells of sensitized animals. This factor promotes an indirect hapten-specific plaque forming response of B cells, irrespective of the identity of the carrier, i.e. provides the Th-2 type of help. Factor production increases with age and occurs most rapidly in strains known to have an accelerated decrease of suppressor capacity. The reason for the inverse correlation between suppressor capacity and the Th-2 type of helper factor is discussed.

Antigens can promote the response to subsequently-administered structurally unrelated macromolecules[4]. In recent yeare the cellular mechanism of this promotion has been elucidated; it has become apparent that there are, in fact, at least 2 types of helper cells. The action of one of these 2 cells depends on a carrier link between the cooperating T and B cells, whereas the other contributes to regulation, irrespective of such a carrier link[5]. Help of this 2nd type, i.e. Th-2 type of help, has been observed in a variety of different inbred strains of mice, and has been shown to increase during adult life and to do so at a rate which varies from strain to strain[6]. The earliest age of maturation was observed in such strains as SJL and MRL/Mp-*lpr*/*lpr*[7], which are known to have defects in generation, amplification or reception of suppressor signals[8–11]. We have been able to link Th 2 type of help with a factor or factors produced in tissue culture in the presence of an antigen with which the cell donors had been previously sensitized[12,13]. Factor-production involves cooperation of nonadherent T cells of primed donors and adherent cells which can be taken from young or old, unprimed donors; the age dependent change in factor is a function of the age at which the donor of nonadherent T cells was primed[13]. In this paper, we shall compare age-dependent changes in the potency of factor production by animals with suppressor defects[9,14–18] and by other animals, in which such defects are relatively small and occur relatively late in life[14,19,20].

Female mice of 5 different strains (SJL, NZB, MRL/MP-*lpr*/*lpr*, A, C57BL/6) were injected i.p. with 100 µg keyhole limpet hemocyanin (KLH) on alum, at various ages. Sensitized animals were sacrificed 8 weeks later; spleen cell suspensions were cultured RPMI 1640 supplement with 5×10^{-5} 2ME, 5% fetal calf serum, streptomycin and penicillin in the presence of KLH for 20 h at 37 °C and cell-free supernatants were collected. Various quantities of supernatants (20, 40 and 60%) were added with dinitrophenylated ovalbumin (DNP_4-OVA) to the assay system (fig. 1). This consisted of suspensions of anti-Thy 1.2 and complement treated spleen cells from female C57BL/6 mice, primed with dinitrophenylated rabbit gamma globulin (DNP_{14}-RGG), eight weeks earlier, when 4 weeks old. Direct and indirect plaque-forming cells, secreting antibody directed against dinitrophenyl (DNP-PFC), were measured, 3 days later. The factor-containing supernatant affected the indirect, but not the direct plaque-forming response and only the latter was used to assess factor activity. To this end, average activity at 40% supernatant content was assessed from values of square root net counts at concentrations of 20, 40 and 60%[13]. Activities were calculated, using, as 100%, the average activity of SJL mice, primed when 16 weeks old.

When SJL animals were sensitized at the age of 4 weeks, factor production was 50% of the adult level. Sensitization of 6–8-week-old animals resulted in the mature level of factor production. A similar rate of development was found in NZB and MRL/Mp-*lpr*/*lpr* (fig. 2; 1st to 3rd panels). The pace of development of the capacity for factor production was markedly slower in A and C57BL/6 mice.

In A mice, factor production was demonstrable after sensitization at the age of 10 weeks, and reached its mature level when animals were sensitized at the age of 32–50 weeks. Factor activity of C57BL/6 animals, sensitized at 4–10 weeks of age, was at the limit of detectability; it reached maximal potency when animals were sensitized at the age of 20 weeks (fig. 2; 4th and 5th panels).

Discussion. Resistance against tolerance induction to aggre-

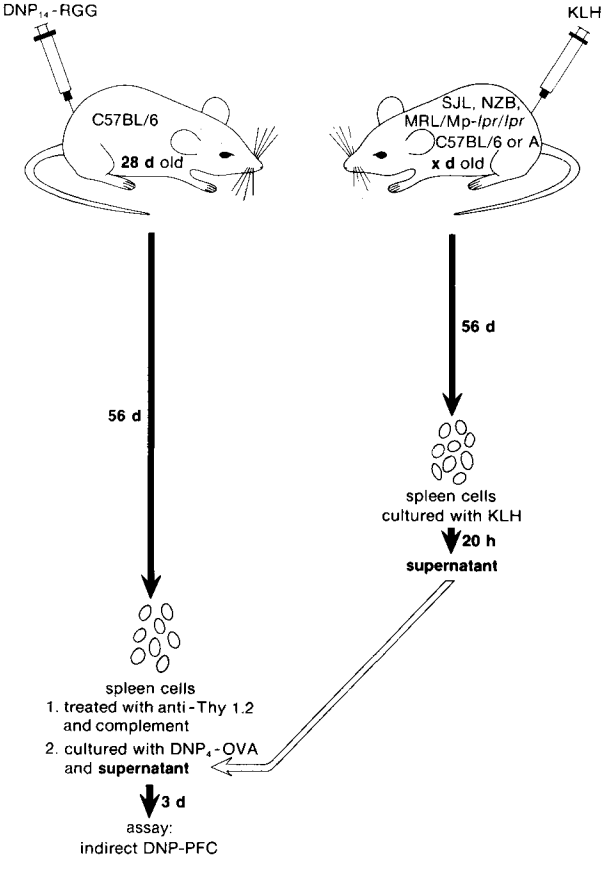

Figure 1. Age-dependent change in a helper factor: experimental design for the production and assay of supernatants, containing a Th-2 factor. Donors were injected s.c. with keyhole limpet hemocyanin (KLH) on alum, or with dinitrophenylated rabbit gamma globulin (DNP-RGG). Supernatants were obtained from spleen cells, cultured in the presence of KLH. These supernatants were added to cultures of spleen cells which had been freed of T cells by treatment with antibody directed against Thy 1.2 and complement; the cultures also contained a haptenated antigen which was conjugated to a carrier other than the one to which the cell donor was sensitized, dinitrophenylated ovalbumin (DNP_4-OVA).

gate-freed RGG is due to defective suppressor capacity in SJL, NZB, MRL/Mp-*lpr/lpr* and BXSB/MpJ[8-11], but not in C57BL/6, where tolerance resistance λ occurs while suppressor capacity undergoes a small and insignificant decrease[20]. Correlation between accelerated capacity to produce Th-2 factor and loss of specific suppressor capacity could be attributable to suppressor capacity decreasing, while helper factor remains constant. This view would be compatible with the fact that loss of suppressor capacity, due to different lesions in the suppressor circuit, correlate with acquisition of Th-2 helper capacity. However, another mechanism may link the 2 processes because 1. admixture of supernatant, devoid of Th-2 activity, from young donors does not diminish the Th-2 potency of active factor[12,13] and 2. treatment with rifamycin SV arrests loss of suppressor capacity without delaying development of Th-2 helper capacity[23]. The inverse correlation between Th-2 and antigen specific suppression could be due to a polymorphism, and an allele which affects a precursor, common to Th-2 and to a crucial link in the suppressor circuit. It remains to be seen whether a differentiation lineage of this type can be demonstrated. However, it remains possible that our experimental conditions did not allow us to produce optimal quantities of suppressor factor and that helper and suppressor cells differ in their sensitivity to rifamycin[23].

It is intriguing to contrast the relatively slow development and the polymorphism of the capacity to develop Th-2 type of help with the age-dependent decline in interleukin-2 (IL-2) and the low capacity for IL-2 production in mice with a defect in the suppressor cell circuit[24-26]. Clearly, the immune balance is in continuous flux and its various components change throughout life, at vastly different rates.

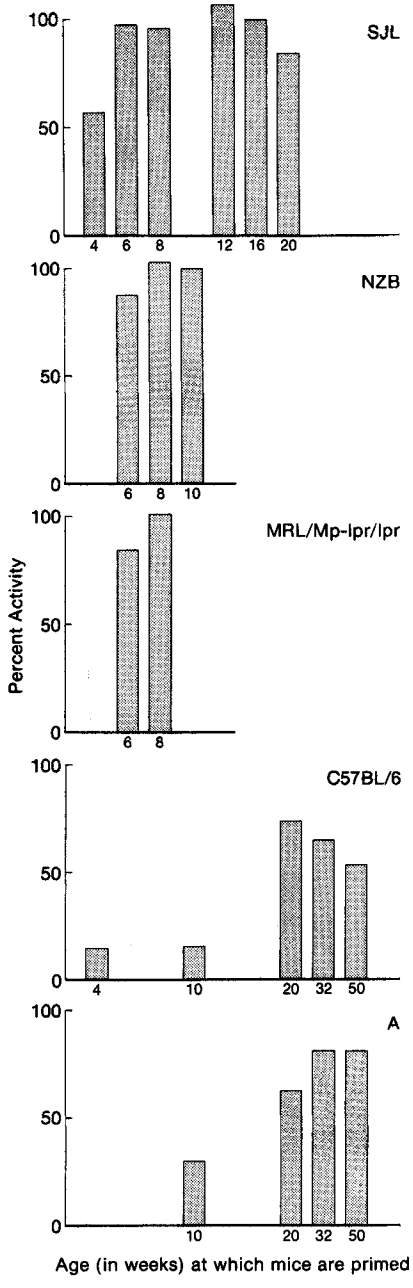

Figure 2. Polymorphism of age-dependent changes of a Th-2 factor. Age, in weeks, at which donors of factors were sensitized, are shown on the horizontal axis. Height of vertical blocks show factor activity as percent; 100% being the factor activity of SJL mice which were sensitized at the age of 16 weeks. The statistical significance of strain differences, allowing for age and concentration, was assessed as $p < 0.01$ (GLIM a statistical system developed by the Royal Statistical Society, distributed by the Numerical Analysis Group, Oxford, U.K.).

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