Brief Definitive Report

IMMUNIZATION WITH ANTIGEN AND INTERLEUKIN 2 IN VIVO OVERCOMES *Ir* GENE LOW RESPONSIVENESS

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Interleukin 2 (IL-2), a lymphokine produced by thymus-derived lymphocytes, has been shown (1, 2) to have a variety of immunologic functions in vitro including an important role in regulating the growth and differentiation of T lymphocytes. It has also been shown (3–5) that in vivo administration of IL-2 can modify immune responses in nude and normal mice. However, the effects of IL-2 on immune responses under control of *Ir* genes have not been tested.

We have been studying the H-2-linked *Ir* gene control of the antibody and T cell responses to sperm whale myoglobin, a model globular protein antigen. Both the T cell proliferative response and the antibody response to myoglobin are controlled in parallel by H-2-linked genes (6, 7). The availability of large amounts of highly purified recombinant IL-2 (8) prompted us to evaluate the influence of IL-2 on immune responses under *Ir* gene control. For this purpose, we immunized low- and high-responder mice with myoglobin together with IL-2, and evaluated its effects on antibody responses. The results show that in vivo administration of IL-2 with myoglobin significantly enhances the antibody response in low-responder mice, to levels similar to those in high-responder mice.

Materials and Methods

Mice. B10.BR/SgSn and B10.D2/nSn were obtained from The Jackson Laboratory, Bar Harbor, ME. B10.D2/nSn *nu/nu* were provided by D. Bolte of the NIH. Small Animal Resources facility. All mice were between 8 wk and 6 mo of age at the time of immunization.

Antigen. The major chromatographic component, IV, of sperm whale myoglobin, purified as described previously (6), was used throughout the studies.

IL-2. The recombinant IL-2 for these studies was kindly supplied by the Cetus Corp. (Emeryville, CA). Homogeneity has been demonstrated by the presence of a single band on sodium dodecyl sulfate (SDS)–polyacrylamide gels with a molecular mass of 15 kilodaltons (8). The endotoxin level in the purified preparations was negligible, <0.1 ng per 10^6 U IL-2, as measured in a standard Limulus assay.

Immunizations and Antimyoglobin Antisera. Mice were immunized with 100 μg of myoglobin in complete Freund's adjuvant with or without IL-2, and bled serially from 10 d to 46 d later. The IL-2 and antigen were emulsified together in the adjuvant.

Enzyme-linked Immunoabsorbent Assay (ELISA) for Antimyoglobin. Concentration of antibodies in the sera was measured by solid-phase ELISA, as described previously (7, 9). Data

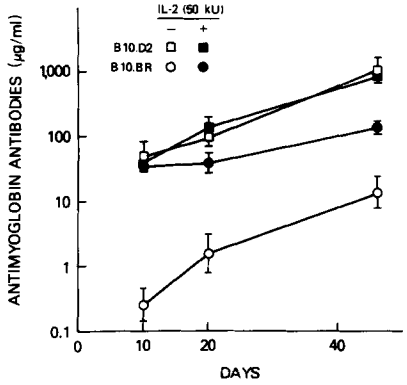

FIGURE 1. Time course of antimyoglobin antibody production by B10.BR (●, ○) and B10.D2 (■, □) mice after an immunization with 100 μg of sperm whale myoglobin, together with 50,000 U IL-2 (closed symbols) or without IL-2 (open symbols). B10.BR is a low-responder strain, and B10.D2 is a congenic high-responder strain to sperm whale myoglobin. Each antiserum was tested individually for the concentration of antimyoglobin antibody by ELISA. Results represent the geometric means and standard errors for five mice of each group. Note logarithmic scale.

obtained from the ELISA were calculated with the log-logit formulation using a program written by Dr. Robert Yarchoan (National Cancer Institute. Bethesda, MD) for a Wang 2200 computer (Wang Laboratories, Lowell, MA).

Results and Discussion

The antibody response and in vitro T cell proliferative response to sperm whale myoglobin are under control of *Ir* genes linked to the mouse H-2 complex (6, 7, 10). To investigate possible effects of exogenous IL-2 on the antimyoglobin response, high- (H-2^d) and low- (H-2^k) responder congenic strains of mice were immunized with sperm whale myoglobin, with or without highly purified recombinant IL-2, and the concentration of antimyoglobin antibodies in the sera was measured. IL-2 was incorporated in the adjuvant emulsion with antigen in order to provide a high local concentration over an extended period.

Figs. 1 and 2 show that the administration of high-dose IL-2 can markedly increase (10–50-fold) the antibody response to myoglobin in low-responder mice, but not in high-responder mice. Without IL-2, the B10.BR mice (H-2^k) showed >10-fold lower response, compared with the B10.D2 mice (H-2^d). In contrast, injection of IL-2 with myoglobin significantly enhanced the antimyoglobin response only in B10.BR mice, and raised antibody levels to the ranges seen in high-responder B10.D2 mice. The enhancement was seen over the entire time course of the response, but no change in kinetics was noted (Fig. 1). The optimum dose of IL-2 was 50,000–100,000 U mixed with the antigen (Fig. 2).

Since the preparation of recombinant IL-2 includes mannitol and SDS, we tested the effect of the vehicle, which has the same amount of mannitol and SDS, on the antimyoglobin response (Fig. 3). Vehicle alone did not increase antibody response in either B10.BR or B10.D2 mice. Also, the influence of possible endotoxin contamination was tested, although endotoxin was not detectable (<0.1 ng in 10^6 U IL-2) in the preparation of IL-2. There was no influence on the antimyoglobin antibody response with the mixture of vehicle and the maxi-

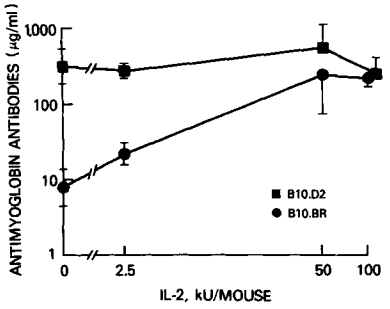

FIGURE 2. Dose-response curve of the effect of IL-2 on antimyoglobin antibody production by B10.BR (●) and B10.D2 (■) mice. Five mice for each group were immunized with 100 μg of sperm whale myoglobin, together with, or without, the indicated dose of IL-2 in Freund's adjuvant, and bled 30 d later. Results represent the geometric means and standard errors for five mice of each group. Note logarithmic scale.

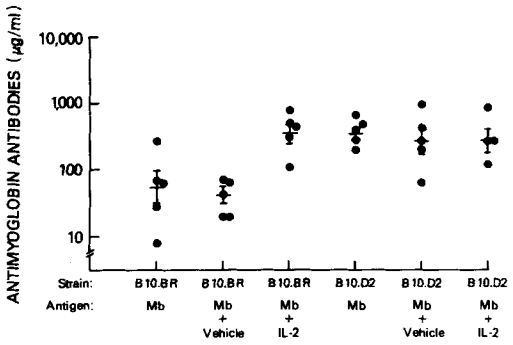

FIGURE 3. The effect of the vehicle used for dissolving IL-2 on antimyoglobin antibody production by B10.BR and B10.D2 mice. Five mice in each group were immunized with 100 μg of sperm whale myoglobin (Mb) together with the indicated reagent. The dose of IL-2 was 25,000 U/mouse. Mice were bled 20 d after the immunization. Each point represents the concentration of antimyoglobin antibody of one mouse, and the geometric means and standard errors for five mice of each group are shown with bars. Note logarithmic scale.

mum possible dose of contaminating endotoxin (1 pg) (data not shown). In addition, IL-2 alone, without myoglobin, did not stimulate B10.BR mice to make antimyoglobin antibodies (data not shown). Thus, this study showed that in vivo administration of IL-2 can increase the magnitude of the antimyoglobin antibody response in low-responder mice.

The mechanism by which IL-2 enhances the antibody response to myoglobin in vivo in low-responder mice is unknown. Since B10.BR mice are low responders, not nonresponders, they do make a low but detectable T cell and antibody response to myoglobin (6, 7, 9, 10). IL-2 may expand the low T cell help, or it may act directly on B cells, as has recently been shown possible in other systems (11–13). To test the latter possibility, we examined the effect of IL-2 mixed with antigen on the antibody response in athymic nude mice. B10.BR nudes were not available, so these experiments were performed with B10.D2 nude mice only. These are congenic to both of the strains used, and are of the high-responder H-2^d haplotype, so they could respond to myoglobin, except for the lack of

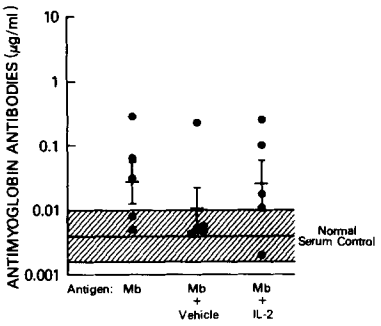

FIGURE 4. The lack of effect of IL-2 on antimyoglobin (anti-Mb) antibody production by B10.D2 nude mice. See Fig. 3 for experimental details. The data in Fig. 3 and Fig. 4 are part of the same large experiment. Therefore, Fig. 3 serves as a positive control for Fig. 4.

mature T cells. When B10.D2 nude mice were immunized with myoglobin with or without IL-2, there was no response and no effect of IL-2 (Fig. 4). The experiment of Fig. 3 was part of the same experiment as Fig. 4, and serves as a positive control. This result shows that the IL-2 effect requires T cells, but does not directly demonstrate that the IL-2 acts on T cells rather than B cells or both.

In contrast, Stotter et al. (3) reported that IL-2 allowed in vivo induction of T helper cells against heterologous erythrocytes in athymic (*nu/nu*) mice. Differences between soluble and particulate antigen, and between recombinant and cell culture IL-2 may explain this discrepancy.

As the response may remain T-dependent even if IL-2 acts directly on B cells to enhance the response, these experiments with athymic B10.D2 nude mice do not exclude the possibility that IL-2 acts directly on B cells in euthymic B10.BR mice. However, given its recognized T cell growth promoting activity, the IL-2 in our experiments probably also acts by expanding the low levels of T cell help known (6, 7, 9, 10) to exist in the low responder. Further, it has been shown (9, 10) that T cells specific for carrier (e.g. fowl γ-globulin) coupled to myoglobin, overcome the *Ir* gene–controlled low responsiveness. Thus, the antibody response to myoglobin controlled by *Ir* genes seems to be dependent on helper T cell activation, as seen in many other studies (14, 15), but not known on B cell repertoire.

Therefore, a reasonable, but not exclusive interpretation of the results is that a low T cell response in vivo in the low responder is enhanced by IL-2, and is reflected in an increased antibody response. In contrast, if the higher T cell response induced by myoglobin in the high responder is not limiting, enhancement of this by IL-2 would not result in an increase in antibody response.

The antibody response to myoglobin in B10.BR mice is normally so low that we have not been able to analyze its fine specificity. Thus, we cannot tell whether the IL-2 enhances responses to epitopes to which B10.BR mice already respond at a low level, or whether it induces responses to new epitopes. Assuming that IL-2, as a nonspecific mediator, affects magnitude of response but not specificity, then its use to amplify the response in B10.BR mice may allow the analysis of their antibody specificity. However, as noted, this assumption may not be valid.

The half-life of IL-2 in the serum is <5 min (5), and thus administration of large amounts are required to demonstrate in vivo effects. The administration of IL-2, together with antigen in the emulsion with Freund's adjuvant, probably

prolongs the local half-life of IL-2 considerably, and thus a relatively high concentration of IL-2 can be maintained locally, where T cells, B cells, and macrophages are responding to antigen.

This is the first report that demonstrates that the administration of IL-2 in vivo, with soluble protein antigen, can increase the antibody response in low-responder mice. Although IL-2 may have been one of the active components of allogeneic effect factor (16), it was not the sole component, since that factor bore major histocompatibility complex antigenic determinants, and had different immunological properties. Concurrently, Durum et al. (17) discovered a similar effect with IL-1. They immunized low-responder mice with poly(Glu_{60},Ala_{30},Tyr_{10}) (GAT), together with IL-1 in emulsion with Freund's adjuvant, and discovered that T cells from those mice proliferated as much as T cells from high-responder mice. In both our and their experiments, lymphokines (IL-1 and IL-2) changed the low-responder phenotype into a high-responder phenotype. In fact, the mechanism of the IL-1 effect may be to induce IL-2, although in the case of GAT, it might also have a contrasuppressor effect (17).

The results of these experiments indicate that the administration of IL-2 is a potential method for manipulating immune responses that are under the control of *Ir* genes. It thus may allow the analysis of the response of low-responder mice more easily, by magnifying small responses. Several studies (18) in humans show that *Ir* genes influence the sensitivity of individuals to vaccination, and that the pathophysiology of some diseases is influenced by *Ir* genes. In clinical use, this method may provide a more effective way to successfully immunize with weak antigens (and possibly tumor antigens), or in immunodeficient patients.

Summary

We studied the effect of purified interleukin 2 (IL-2), made by recombinant DNA techniques, on the serum antibody response to myoglobin in high- and low-responder mice. Previous studies (6, 7) have shown that this response is controlled by H-2-linked *Ir* genes. The IL-2 was emulsified with the antigen in complete Freund's adjuvant to provide a sustained high local concentration. In low-responder B10.BR mice, a single dose (optimum 50,000 U) resulted in a consistent 10–50-fold increase in specific serum antibody throughout the time course of the response, from 10 d to 46 d after immunization. In contrast, no effect of IL-2 was seen in congenic high-responder B10.D2 mice. With IL-2, the low-responder mice achieved specific antibody levels comparable to those of high responders. Vehicle alone had no effect, and IL-2 alone, without antigen, did not induce myoglobin-specific antibody. No effect of IL-2 was seen in athymic nude mice of high-responder H-2 haplotype. The effect of IL-2 may be on a small number of responding T cells in the low responder mice, but it is possible that IL-2 also acts directly on B cells in a response that remains T-dependent, and therefore is not observed in athymic mice. We suggest that IL-2 may enhance suboptimal T cell help in the low responder, whereas help is not limiting in the high responder. This approach may enable the study of antibody responses in low responders otherwise too weak to analyze, and may be useful in producing antibodies to poorly immunogenic antigens. Potential clinical uses include immunization with weak antigens in normal patients, or with any antigen in certain immunodeficient patients.

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