

Cyclophosphamide and Interleukin-12 Synergistically Upregulate the Acquisition of Allergic Contact Dermatitis in the Mouse

HENRY C. MAGUIRE, Jr.

Departments of Medicine and Dermatology, Thomas Jefferson University, Philadelphia, USA

Cyclophosphamide given before allergen and recombinant interleukin-12 administered at the time of allergic sensitization substantially increase the acquisition of allergic contact dermatitis in the mouse. Since their immunoadjuvant mechanisms appeared different, it seemed probable that combining cyclophosphamide pretreatment with interleukin 12 administration would result in a more intense allergic contact dermatitis than when either agent was used alone. This was tested in different groups of mice sensitized to dinitrofluorobenzene or to oxazolone. Consistently, immunopotentialization of allergic contact dermatitis was significantly greater with the two immunoadjuvants than with either alone. This immunoadjuvant combination is likely to find use in immunization protocols designed to induce a Th-1 helper cell response. **Key words:** *delayed type hypersensitivity; immunopotentialization; Th-1 cells; immune adjuvant.*

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H. Maguire, Department of Medicine (Neoplastic Diseases), Room 1024, Curtis Building, Thomas Jefferson University, 1015 Walnut Street, Philadelphia, PA 19107, USA.

In past work we have identified a number of immunomodulators that upregulate the acquisition of allergic contact dermatitis (ACD) including cyclophosphamide (Cy) pretreatment, *C. parvum* (*P. acnes*), interferon gamma and interleukin-12 (IL-12) (1–5). In the case of immunopotentialization with Cy, it appears that the immunomodulation turns on the selective toxicity of that alkylating agent for precursors of cells that, in response to antigen, will develop specific suppressor activity (6, 7). These suppressor cells appear to be a subset of T cells; their exact nature and function is not certain and is a matter of continuing debate. However, the phenomenon of Cy up-regulation of ACD is well-recognized and has been demonstrated in a variety of experimental animals and in man (8–10).

IL-12 was discovered as a secretory factor of Epstein-Barr transformed human B cells. This factor activated natural killer cells and originally was called natural killer cell activating factor. In another context, the same factor was found to enhance the activity of cytotoxic T cells (11). The genes for both human and murine IL-12 have been cloned and the recombinant products expressed. In the context of regulation of T cell reactions, it has been found that, following immunization, IL-12 favours the development of helper cells of the Th-1 phenotype (interferon gamma, interleukin-2 secreting) and inhibits the development of Th-2 helper cells (IL-4 and IL-5 secreting) (12). The specific effector cells of delayed type hypersensitivity (including ACD) appear to be Th-1 helper T cells.

We hypothesized that, with apparently different mechanisms, the immunoadjuvant effects of Cy and of IL-12 might complement one another. We have examined this issue in the mouse model of ACD.

MATERIALS AND METHODS

Animals

Balb/cJ and Balb/ByJ female mice were purchased from the Jackson Laboratories (Bar Harbor, Maine, USA) and studied at 8–12 weeks of age. The mice were kept in a temperature-controlled, light-cycled room in our laboratory animal facility.

Chemicals

Murine recombinant IL-12 was donated by Genetics Institute (Cambridge, MA, USA). Chinese hamster ovary cells were cotransfected with the cDNAs of the two peptides (p35 and p40) that constitute the IL-12 heterodimeric molecule. The final product had a specific activity of 5.6×10^6 U/mgm and lacked detectable endotoxin activity by the Limulus amoebocyte assay. In different experiments, the individual dose of IL-12 was 1–1.5 µg contained in 0.1 ml vehicle; the vehicle was 1% bovine serum in saline. Fresh stocks of Cy, of dinitrofluorobenzene (DNFB) and of 4-ethoxymethylene-2-phenyloxazolone (oxazolone) were purchased from Sigma (St. Louis, MO, USA). The Cy was administered intraperitoneally at a dose of approximately 50 mgm/k contained in 0.1 ml saline.

Sensitization and challenge

Mice were clipped on a rear dorsal flank and sensitized by the application of 20 µl of 1% DNFB or 5% oxazolone in a vehicle consisting of four parts acetone and one part corn oil. Challenge was made on an ear by the application of a total of 5–7 µl of DNFB (0.1% or 0.2%) or oxazolone (0.1%) to both surfaces of the distal pinna following the method originally described by Asherson & Ptak (13). Baseline and later measurements of ear thickness were made using a pressure-sensitive engineer's micrometer.

Statistics

The Mann-Whitney Test was used, choosing a level of significance of $p < 0.05$.

RESULTS

In a representative experiment, four groups of five Balb/c mice were sensitized to DNFB on Day 0. Groups 1 and 2 received 2 mgm Cy intraperitoneally one day before sensitization (Day –1). Groups 1 and 3 were given 1.5 µg IL-12 intraperitoneally on Day 0. Group 4 mice were sensitized without Cy or IL-12. Ear challenge was made on Day 10 with 0.1% DNFB to the sensitized mice (Groups 1–4), as well as to naive Balb/c mice (Group 5). The results are shown in Fig. 1.

Cy or IL-12 alone resulted in significant immunopotentialization only at the 2 day point, whereas combined Cy/IL-12 gave increases in the strength of the challenge reactions that were substantially higher than the other groups at both the 1 day and 2 day points ($p < 0.05$). We have repeated this experiment with DNFB two times, with similar results. Specimens taken of challenge reactions from mice sensitized to DNFB with Cy plus IL-12 immunopotentialization showed a histology marked by edema and a round cell infiltrate that was typical of ACD in the mouse (data not shown). Lack of significantly increased contact reactions at 1 day in mice sensitized using a single

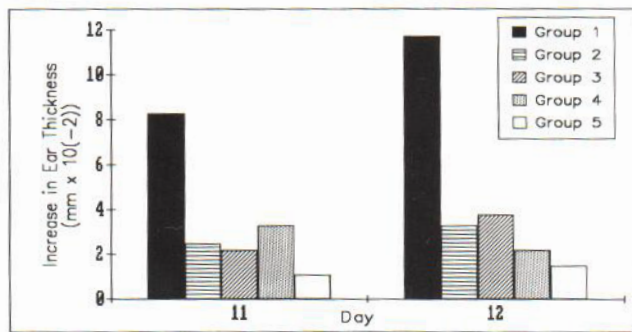


Fig. 1. Immunopotentiality of DNFB allergic contact dermatitis by Cy and IL-12. Groups of mice were treated with immunomodulators as follows: Group 1: Cy and IL-12; Group 2: Cy, Group 3: IL-12. Groups 1-4 were DNFB-sensitized on day 0 and ear challenged on day 10 in parallel with naive mice (Group 5). The day 11 and day 12 average increases in ear thickness over baseline for each group are shown in the bar graph. At 24 and 48 h, group 1 > 2,3,4, $p < 0.05$.

dose of IL-12 at the time of sensitization is sometimes seen; several days administration of IL-12 at the time of sensitization gives more consistent heightening of the 24-h ACD responses.

We tested whether the immunopotentiality with Cy plus IL-12 would hold for an unrelated sensitizer, oxazolone, and whether the sensitivity would persist. Four groups of five Balb/c mice were contact-sensitized to oxazolone on Day 0. Groups 1 and 3 received Cy (2 mgm) on Day -1. Groups 1 and 2 were given IL-12 (1.1 µg) intraperitoneally on Day 0 and Day 1. Group 4 received oxazolone but no Cy or IL-12.

Challenge was made to the right ear with 7 µl of 0.1% oxazolone on Day 5 and to the left ear on Day 13. Different naive toxicity control groups were included at the first and second testing (Groups 5 and 6, respectively). The results are shown in Fig. 2. At first testing, the combination of Cy plus IL-12 more efficiently immunopotentiality than either immune modulator alone. We performed a second challenge, on the opposite ear, a week after the first testing. The mice sensitized with immunoadjuvants continued to have strong ACD reactiv-

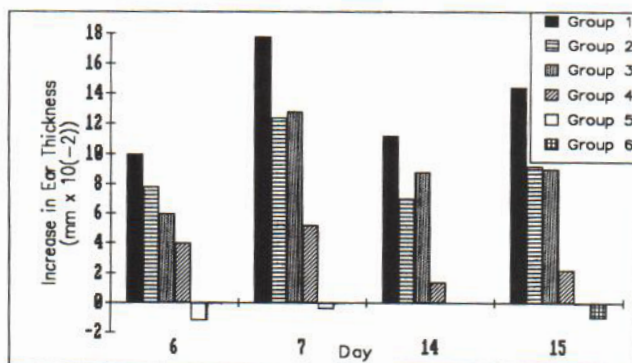


Fig. 2. Persistence of allergic contact dermatitis to oxazolone induced with Cy and IL-12. Groups of mice were treated with immunomodulators as follows: Group 1: Cy and IL-12; Group 2: IL-12; Group 3: Cy. Groups 1-4 were sensitized with oxazolone on day 0 and challenged on day 5 (right ear) and on day 13 (right ear). Groups 5 and 6 were naive toxicity control mice. The one and two day average increases in ear thickness over baseline for each group are shown in the bar graph. Day 5 testing: at day 6 group 1 > 3, 4 $p < 0.05$; at day 7, group 1 > 2,3,4 $p < 0.05$. Day 13 testing: at day 14 and day 15, group 1 > 2,3,4 $p < 0.05$.

ity, whereas the intensity of the reactions in the conventionally sensitized mice was considerably reduced. Such reductions of contact hypersensitivity are characteristic of ACD induced without adjuvant in mice (14).

DISCUSSION

IL-12 is a pleotropic immune modulator. Given at the time of first exposure to antigen, it heightens the Th-1 response and suppresses the Th-2 response. In the case of certain diseases like Leishmaniasis in a mouse model, in susceptible mice the administration of IL-12 early in the infection causes the disease to be self-limited and to ultimately resolve, whereas without IL-12 it is progressive and ultimately lethal (15).

While the immunomodulatory effect of IL-12 on the induction of ACD is substantial, the same IL-12 treatment given at the time of challenge consistently fails to up-regulate the skin test response (data not shown). In our study, the naive control mice did not receive any immunomodulators. Consequently, we have not formally excluded the possibility that the increased ear reactions to challenge with sensitizer represented a significant lowering of the irritant reactions in the test animals by Cy or IL-12. Such a phenomenon is not suggested by the literature and we feel it to be unlikely. However, we will test the issue experimentally in future experiments.

Histologically the ACD reactions in mice sensitized with IL-12 were comparable to the ACD reactions of conventionally sensitized mice and did not suggest immediate type hypersensitivity reactions. Measurements of ear thickness 2 h after challenge in mice sensitized using, for immunopotentiality, IL-12 and/or Cy revealed no significant change over baseline.

It is likely that IL-12 can usefully be incorporated into protocols for evaluating the allergenicity of prospective sensitizers. While it has not been tested directly, it is probable that IL-12 will up-regulate the immunogenicity of weak contact allergens, just as it does the immunogenicity of the strong allergens DNFB and oxazolone.

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