

Short Communication

Effects of Interleukin 4 on Production of IgE Monoclonal Antibodies Directed against Glucose Oxidase

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ABSTRACT

Interleukin 4 is a cytokine that promotes the growth of B cells and the synthesis of IgE subclass antibody. Here we report the results of our study that was designed to determine if recombinant murine interleukin 4 (rmIL-4) can increase the yield of hybridomas secreting IgE subclass monoclonal antibodies directed against glucose oxidase. Three BALB/c mice (test group) were primed i.p. with 50 μ g of glucose oxidase and then boosted i.v. 2 weeks later with 5 μ g of glucose oxidase plus 200 ng of rmIL-4. A standard hybridoma fusion was performed 3 days later, and the fusion products were then cultured in the presence of 20 ng of rmIL-4 per milliliter. Another group of three mice underwent an identical immunization and fusion procedure, except that the rmIL-4 was omitted (control group). At 21 days after fusion, the test group had a significantly greater frequency of wells with hybridomas (average frequency = 191 per 1000 wells in test group versus 58 in control group; $\chi^2 = 1803$; $p = 0.0001$) and wells containing IgG anti-glucose oxidase (mean of 23 per 1000 wells in test group versus 3 in control group; $\chi^2 = 506$, $p = 0.0001$). Moreover, the IL-4-treated group had a significantly higher frequency of wells containing IgE antibodies (mean of 44 versus 11 in control group; $\chi^2 = 288$, $p = 0.0001$) and IgE antibodies directed against glucose oxidase (mean of 5 per 1000 wells versus 2 in control group; $\chi^2 = 21$, $p = 0.0001$). We conclude that rmIL-4 significantly increased the yield of hybridomas secreting IgG and IgE monoclonal antibodies directed against glucose oxidase.

THE PRODUCTION of IgE monoclonal antibodies is of potential interest to cancer investigators because IgE antibodies have been shown to be capable of inhibiting the growth of murine mammary carcinoma *in vivo*.⁽¹⁾ Moreover, eosinophils have high-affinity receptors for IgE,⁽²⁾ and eosinophils can infiltrate and kill a wide variety of tumors.⁽³⁾ Thus, IgE and eosinophils armed with anti-tumor IgE theoretically could have value for the immunotherapy of some tumors.

Interleukin 4 (IL-4) is a cytokine that promotes B cell growth and the synthesis of IgE subclass antibody.⁽⁴⁾ To date, there has been only one published report describing the use of IL-4 to generate IgE monoclonal antibodies,⁽⁵⁾ and that study utilized Epstein-Barr virus-infected human B cells. In this study, we hypothesized that recombinant murine IL-4 (rm IL-4) could also be used to increase the yield of mouse hybridomas that secrete IgE antibody against a fungal flavoenzyme, glucose oxidase.

We selected glucose oxidase as the target antigen because our laboratory has been investigating the use of this enzyme for the treatment of certain lymphomas.^(6,7) In addition, glucose oxidase generates hydrogen peroxide under physiological conditions, and eosinophil peroxidase utilizes hydrogen peroxide as a substrate to produce highly cytotoxic hypohalous acid. Thus, an IgE antibody directed against glucose oxidase could conceivably be used to target eosinophils to a catalytic generator of substrate for eosinophil peroxidase.

Three pairs of female, BALB/c mice (Simonson Laboratories, Gilroy, CA), 10–12 weeks old, were used in this study. Each pair of mice received an intraperitoneal (i.p.) primer immunization consisting of 50 μ g of glucose oxidase (Boehringer-GmbH, Mannheim, Germany) emulsified in 200 μ l of phosphate-buffered saline (PBS) and Freund's complete adjuvant (Sigma, St. Louis, MO). Two weeks after priming, one

mouse in each pair received an intravenous (i.v.) booster injection consisting of 5 µg of glucose oxidase in 200 µl of PBS (control mouse). The other mouse (test mouse) received an identical boost that also contained 200 ng of rmIL-4 (Genzyme, Cambridge, MA).

Three days after boosting, the spleens from both mice were harvested, and standard hybridoma fusions were performed,⁽⁸⁾ using the Sp2/0-Ag14 myeloma cell line (American Type Culture Collection [ATCC], Rockville, MD) cultured in complete Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Hybrimax; Sigma), 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, and 1 mM sodium pyruvate. Cell fusions were performed with high molecular weight polyethylene glycol (Sigma) and a 1:1 spleen:tumor cell ratio. Following fusion, the cells were plated into individual wells of up to ten 96-well microculture plates. The fusion products from the test mice were then cultured in hypoxanthine-aminopterin-thymidine (HAT) medium containing rmIL-4 (20 ng/ml), whereas the cells from the control mice were grown in HAT medium alone.

At 21 days after fusion, the plates were examined visually by inverted microscopy, and the number of wells showing hybridoma growth was recorded for each fusion. For statistical analysis using the χ^2 test, we normalized the numbers from each fusion to "hybridoma frequency per 1000 wells" (Table 1). We then considered the "expected" frequency for each pair of fusions to be the result obtained in the absence of IL-4 (control mouse) and the "observed" frequency to be the result obtained in the IL-4-treated fusion. As demonstrated in Table 1, there was a highly significant increase in the frequency of hybridomas generated after IL-4 treatment.

Each well showing growth was then tested in an enzyme-linked immunosorbent assay (ELISA) for the presence of IgG anti-glucose oxidase. In brief, individual wells of polystyrene microtiter ELISA plates were coated with glucose oxidase (20 µg/ml) in PBS and then blocked with 1% bovine serum albumin. Hybridoma supernatants (100 µl) were then incubated in each well for 90 min at room temperature. Following two wash steps, bound mouse IgG was detected using 1 µg/ml of rat anti-mouse κ light chain antibody conjugated to horseradish peroxidase (Zymed, South San Francisco, CA). The positive control in each assay was 5 µg/ml of mouse IgG monoclonal antibody to

glucose oxidase (Zymed). The negative control was a pentuplicate assay using normal mouse IgG at 5 µg/ml.

2,2'-azino[3 ethylbenzthiazoline-6-sulfonic] acid (ABTS) (Bio-Rad, Richmond, CA) was used as the substrate for the peroxidase-conjugated detecting antibody, and the optical density at 415 nm in each well was measured with a Bio-Rad model 450 ELISA plate reader. An assay was considered valid if the positive control in each assay yielded an optical density of at least 0.2, and the mean of the optical densities of the negative controls was less than 0.09. A test well was considered positive if its optical density was more than 2 standard deviations above the mean of the pentuplicate negative controls. The data are shown in Table 1, and it is apparent that the frequency of hybridomas secreting IgG anti-glucose oxidase was significantly greater in the fusions that were treated with rmIL-4.

Similarly, each well showing hybridoma growth was also tested by ELISA for the presence of IgE antibody after an additional 5 days of growth. For this assay, the capture antibody was monoclonal rat anti-mouse IgE (Zymed) used at a concentration of 1 µg/ml. The positive control was 1 µg of purified mouse IgE per milliliter (ICN, Costa Mesa, CA) and the detecting antibody was peroxidase-conjugated rat anti-mouse κ light chain antibody, as described above.

The results of the IgE ELISA are listed in Table 2. Once again, treatment with rmIL-4 yielded a significantly greater frequency of wells containing IgE than fusions performed without rmIL-4.

Finally, we retested the supernatants from the wells containing IgE antibodies in order to detect IgE antibodies directed specifically against glucose oxidase. First, the wells of the ELISA plate were coated with the capture antigen, glucose oxidase, and then blocked with bovine serum albumin. Following incubation of each well with hybridoma supernatant, IgE bound to the glucose oxidase was detected with rat anti-mouse IgE antibody (Zymed) conjugated to activated horseradish peroxidase (Zymed) in accordance with the manufacturer directions. A supernatant was considered to be positive if it produced an optical density more than two standard deviations higher than the mean of the negative control (triplicate assays of purified mouse IgE used at a concentration of 5 µg/ml).

When tested by this assay, the hybridoma supernatants from the various fusions yielded relatively small numbers of positive

TABLE 1. COMPARISON OF FREQUENCIES OF HYBRIDOMAS AND IgG ANTI-GLUCOSE OXIDASE ANTIBODIES WITH OR WITHOUT RECOMBINANT MURINE INTERLEUKIN 4 TREATMENT

Hybridoma frequency/1000 wells ^a		IgG-Anti-GO/1000 wells ^a	
Observed (with IL-4)	Expected (no IL-4)	Observed (with IL-4)	Expected (no IL-4)
87	79	4	1
289	44	46	4
198	50	19	4
Mean: 191	58	23	3
$\chi^2 = 1803$ $p = 0.0001$		$\chi^2 = 506$ $p = 0.0001$	

^aNumbers in each column refer to the normalized frequency of the event per 1000 wells of each of 3 paired fusions.

TABLE 2. COMPARISON OF FREQUENCIES OF HYBRIDOMAS SECRETING IgE AND IgE ANTI-GLUCOSE OXIDASE

Wells with IgE/1000 wells ^a		IgE-Anti-GO/1000 wells ^a	
Observed (with IL-4)	Expected (no IL-4)	Observed (with IL-4)	Expected (no IL-4)
27	7	2	1
81	21	8	2
25	6	4	2
Mean: 44	11	5	2
$\chi^2 = 288$ $p = 0.0001$		$\chi^2 = 21$ $p = 0.0001$	

^aNumbers in each column refer to the normalized frequency of the event per 1000 wells of each of 3 paired fusions.

results (Table 2). Nevertheless, those fusions that included rmIL-4 consistently produced a higher frequency of IgE antibodies against glucose oxidase than the control fusions, and the results were once again statistically significant by the χ^2 test. When these IgE-secreting hybridomas were then expanded and cloned by limiting dilution, the clones continued to secrete IgE subclass antibodies for the duration of subsequent culturing (6 weeks).

On the basis of these results, therefore, we conclude that rmIL-4 significantly increased the frequency of hybridomas that produced IgG and IgE antibodies against glucose oxidase. It remains to be determined, however, if the protocol that we utilized is the optimum one for generating IgE monoclonal antibodies, and further studies will be needed in order to validate the utility of this procedure for other antigens. Nevertheless, we propose that our findings will be of potential interest to other investigators who seek to produce IgE monoclonal antibodies for use in various experiments.

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