in vivo *18*: 649-652 (2004)

Interleukin 4 Increases the Antibody Response Against Rubisco in Mice

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Abstract. *The influence of interleukin 4 (IL-4) on antibody titer in serum and spleen culture supernatant in mice immunized with spinach (Spinacia oleracea L.) Rubisco was investigated. Therefore, we boosted one mouse additionally to the antigen with recombinant mouse IL-4. We found that the Rubisco-specific antibody titer in serum as well as in spleen cell culture supernatant was significantly enhanced in the IL-4 mouse. Most of the antibodies were of the IgG1 subclass. After hybridoma generation, Rubisco-specific antibodies were found in more than 95% of the wells tested compared to about 12% of the control mouse.*

Several cytokines enhance the growth, differentiation and maturation of B lymphocytes (1). Especially, interleukin 4 (IL-4) is known to intensify B cell growth and also to promote the synthesis of IgE subclass antibodies (2). It was first described in 1982 as a cofactor in the proliferation of resting B cells (3). The following effects of IL-4 were shown in experiments done later on: a) activation of resting B cells, b) promotion of growth of B cells by driving DNA replication after antigen stimulation, c) stimulation of class switch to IgG1 and IgE antibodies in proliferationg B cells (4, 5).

In this report, we investigated the influence of IL-4 on antibody titer in serum and spleen culture supernatant when applied together with the boost injection of an antigen. As a model antigen we used Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase, EC 4.1.1.39) from spinach (*Spinacia oleracea L.*).

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Key Words: Boosting, hybridoma, immunization, interleukin 4, spleen culture supernatant, Rubisco.

Materials and Methods

Two female four-month-old Balb/c mice were intraperitoneally immunized according to the following schedule: 1st injection of 250 μg Rubisco (Calbiochem, Bad Soden, Germany) in 150 μl phosphate-buffered saline (PBS) with an equal quantity of Freund's complete adjuvant (CFA) (6); after one month, 2nd injection of 250 μg Rubisco (Calbiochem) in 150 μl PBS without adjuvant; 10 months later, 3rd injection of 70 μg highly purified Rubisco (a kind gift of Eiichi Mizohata, Osaka, Japan) (7) in 100μl PBS; and two weeks later, 4th injection of 25 μg highly purified Rubisco in 100 μl PBS. One mouse received an additional *i.p.* injection of 200 ng recombinant mouse IL-4 (Biotrend, Cologne, Germany) (5).

Four days later, the spleen cells from both mice were fused with X63-Ag8.653 myeloma cells using standard electrofusion technique. Briefly, the spleen/myeloma cell ratio was about 3:1 and the fusion was performed in 2 mm cuvettes using 900V, 5 ohms, 100 μF and 20 μs in an electrofusion device (Dr.L.Fischer, Heidelberg, Germany) . Following fusion, about 100,000 cells per well were plated into eight 96-well plates (Nunc, Wiesbaden, Germany) per mouse and cultured in hypoxanthine-aminopterin-thymidine (HAT) medium containing either 10 ng/ml IL-4 or no interleukin. As an additional control, non-fused spleen cells were cultured in RPMI 1640 medium containing 10% fetal calf serum without IL-4. Serum of both immunized mice was also included in the experiment.

Two weeks after the fusion, the plates were examined visually and clones, serum and spleen culture supernatant were tested in enzyme-linked immunosorbent assay (ELISA) as follows: highly purified spinach Rubisco (5 μg/ml in PBS) was adsorbed to the solid phase of microtiter plates overnight at 4°C. After blocking with PBS containing 5% neonatal calf serum (PBS/NCS), the plates were incubated with culture supernatants or serum in different dilutions in PBS/NCS for 60 min at room temperature. After washing with tap water, either polyclonal goat anti-mouse-Ig-POD (peroxidase) conjugate, goat anti-mouse-IgG-POD conjugate (Sigma) or class- and subclass specific biotin-conjugated goat anti-mouse Ig followed by peroxidase-conjugated streptavidin (Roche Diagnostics, Mannheim, Germany) was added and incubated for 60 min at room temperature. After washing, the test was developed with ortho-phenylendiamine (OPD) and H_2O_2 and optical density was measured at 490nm in an ELISA plate reader. Values less than 0.3 were calculated as negative.

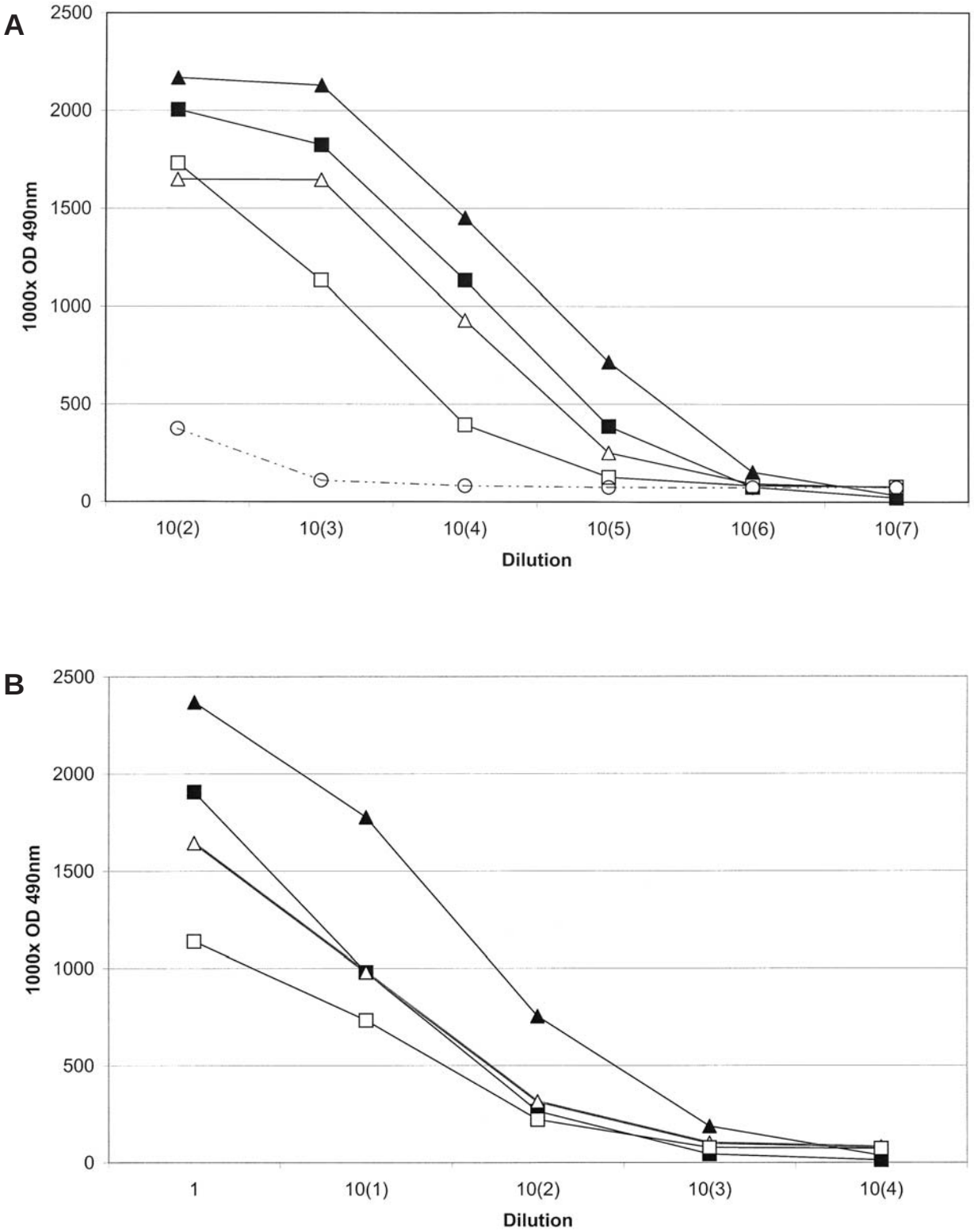

Figure 1. *Antibody reactivity in sera (A) or spleen cell culture supernatants (B) of immunized mice against spinach Rubisco in ELISA. Antibodies were detected either with goat anti-mouse-Ig (full symbols) or goat anti-mouse-IgG (open symbols). Triangles (▲) represent samples from the mouse treated with Rubisco plus IL-4 and squares (■) represent samples from the mouse immunized without IL-4 addition. The open circles (*O*) represent the Balb/c mouse normal serum.*

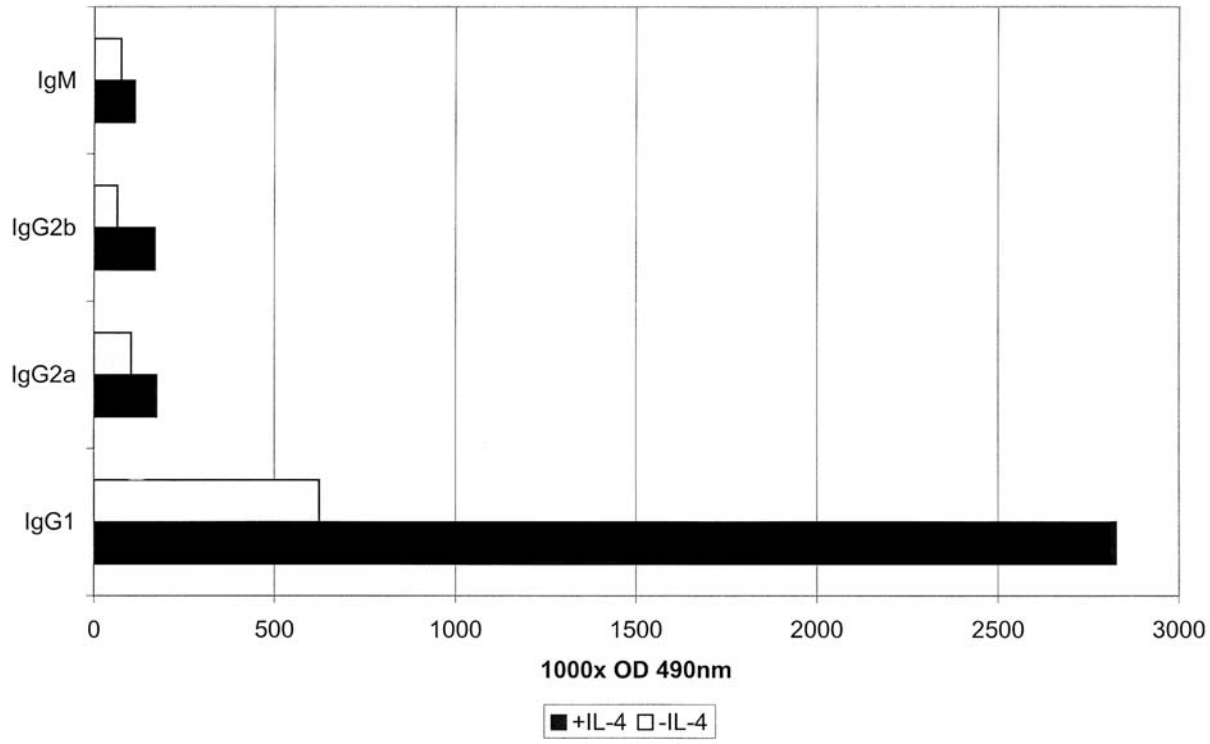

Figure 2. *Subclasses of anti-Rubisco antibodies in spleen cell culture supernatants from mice boosted with or without IL-4.*

Table I. *Percentage of positive (strongly-positive in parentheses) wells tested in ELISA against Rubisco. The culture medium contained 10ng/ml IL-4 or no IL-4.*

	Mouse with IL-4	Mouse without IL-4
Medium with IL-4	99% (94%)	20% (1%)
Medium without IL-4	92% (20%)	3% (-)

Results and Discussion

The visual examination of the fusion plates showed up to ten clones per well in the plates from the mouse boosted with IL-4. The mouse without IL-4 treatment had about 10 to 20 clones per 96-well plate, the general quantity of a normal fusion. When testing for specificity against Rubisco, we found that almost all wells from the IL-4 mouse contained anti-Rubisco antibodies (94% with strongly positive reaction), suggesting that at least one clone per well would produce an antibody against Rubisco. Almost the same result was achieved when the fused cells were cultured in medium lacking additional IL-4. From the non-IL-4 mouse, we obtained 9 positive wells out of 384 tested. The number of positive wells increased to 20% when IL-4 was added to the medium. The results of the fusion are summarized in Table I. The serum of both mice was highly reactive against spinach Rubisco; dilutions of up to 10^5 were strongly-positive (Figure 1A). The serum titer of the IL-4 mouse was considerably higher than that of the control mouse. This was also observed for the IgG response.

Spleen culture supernatants from the IL-4 mouse contained many more specific antibodies, resulting in an 80% higher ELISA signal compared to the culture from the mouse immunized according to the standard protocol (Figure 1B). This difference could also be observed in IgG content. When testing for subclass distribution (8), we found a significantly higher titer of IgG1 antibodies in the spleen culture supernatant of the IL-4 mouse, suggesting that IL-4 enhances the class switch to IgG1 (Figure 2). This effect was described for IL-4 transgene mice in 1991 (9), when these mice showed enhanced levels of IgG1 and IgE.

Summarizing, we found that boosting of mice together with IL-4 increases the amount of antigen-specific antibodies in serum and in spleen culture supernatant. This might be due to the longer surveillance of non-fused splenocytes in culture. A higher percentage of antibodies of

the IgG1 subclass was also obtained, which is a great advantage because they are easier to handle and to purify. There was also a considerable increase in the production of antigen-specific hybridomas. The addition of IL-4 to the immunogen could therefore make it possible to produce higher quantities of hybridomas. This would be especially advantageous in cases where the production of monoclonal antibodies is difficult. For the production of polyclonal antibodies *e.g.* anti-peptide and anti-Ig antibodies, or the long time maintenance of T-cell clones (10), the addition of IL-4 could also be extremely useful.

Acknowledgements

We thank Eiichi Mizohata, Osaka University, Japan for sending us purified spinach Rubisco. The work was supported in part by a BioChance grant from BMBF.

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Received June 15, 2004
Accepted August 6, 2004