



STUDIES ON THE IMMUNOGLOBULIN-E SYSTEM OF THE COMMON MARMOSET IN COMPARISON WITH HUMAN DATA

Steffen Schmidt, Reinhard Neubert*, Marcus Schmitt*, Diether Neubert

University Medical Center, Free University Berlin
Institute of Toxicology and Embryopharmacology, and
*Children's Hospital (Kaiserin Auguste Victoria Haus)

(Received in final form June 17, 1996)

Summary

In the common marmoset (*Callithrix jacchus jacchus*) immunoglobulin E (IgE) serum levels and IgE synthesis of peripheral blood mononuclear cells (PBMC) *in vitro* were investigated in order to look for homologies to the human system. While IgE was not found in marmoset blood plasma with three commercial antihuman IgE-kits with monoclonal antibodies (mAbs), two other kits using polyclonal antibodies against human IgE revealed detectable IgE concentrations of up to 10 kU/liter in plasma samples of 19 out of 21 marmosets. In accord with human data, *rhIL-4* showed biological functions under *in vitro* conditions in PBMC of the New World monkey. Proliferation, measured by ³H-thymidine incorporation, of isolated PBMC of marmosets could be induced by *rhIL-4*. FACScan analysis showed an enhanced expression of the low affinity IgE receptor CD23 (FcεRII) on CD20⁺ B lymphocytes after incubation with *rhIL-4*. Furthermore, PBMC from marmosets could be stimulated by IL-4 alone or in combination with dexamethasone as well as with lipopolysaccharide (*E. coli*) to produce IgE in culture. The results indicate that *Callithrix jacchus* is using an IgE system that is rather similar to that of humans, although not completely identical. Antihuman mAbs and *rhIL-4* can be used to investigate IgE regulation *in vitro* of marmoset PBMC. These data encourage the development of a primate animal model for studying possible modifications of the IgE system under pathological conditions to find new therapeutic strategies in atopic diseases.

Key Words: marmosets, *Callithrix jacchus*, immunoglobulin E, IgE, CD23, IL-4

The New World monkey *Callithrix jacchus* has been shown (1-4) to represent a convenient model for studying effects on defined immunological components, such as white blood cell surface receptors, because of pronounced cross-reactivities (5) with antihuman monoclonal antibodies (mAbs), although some differences in immunological components between humans and marmosets have been recognized. Until now, only little information is available on functional aspects of the immune system in this species, particularly on the IgE system.

In the last few years it became clear that IgE synthesis in humans requires two different signals. The first is delivered by IL-4, a cytokine mainly produced by type-2 helper lymphocytes. IL-4 induces isotype switching through activation of the IgE heavy chain transcription in B cells, leading to an epsilon germ-line transcript (6). To generate the intact IgE heavy chain, the second signal is necessary. *In vitro* studies with human blood and *in vivo* studies in mice have shown that this signal *in vivo* is given by cognate T/B cell interaction, and it can be induced with Epstein Barr virus, lipopolysaccharide, anti-CD40 antibodies or glucocorticoids *in vitro*.

In this communication we report on comparative studies to reveal similarities as well as possible differences within the IgE system of marmosets, as compared to the well-known human IgE system. It was investigated whether IgE can be detected in the blood plasma of marmosets with commercial antihuman antibodies. Furthermore, it was evaluated whether this system in the marmoset responds in a similar way to human recombinant interleukin-4 (*rhIL-4*) and to a number of stimuli such as dexamethasone and lipopolysaccharide (*E. coli*), as does the corresponding system in humans. Special emphasis was put on the analysis of both the stimulation of IgE synthesis *in vitro* and the expression of CD 23, the low-affinity receptor (FcεR) for IgE.

Materials and Methods

Maintenance of marmosets and blood sampling: Healthy adult marmosets (weight > 300 g) were bred and maintained in our colony in Berlin at a temperature of $25 \pm 1^\circ\text{C}$ and $55 \pm 5\%$ relative humidity and a defined light/dark cycle (light from 6 a.m. to 6 p.m.). They received marmoset pellet feed (Altromin®), water ad libitum and additionally apples, carrots, bananas and boiled eggs. A multivitamin preparation (including vitamin D₃) and calcium were applied orally every two weeks.

From the marmosets, blood (up to 2 ml) was drawn from the femoral vein plexus in intervals of at least 4 weeks. Clotting was prevented by adding 50 IU of heparin ("stabilizer-free", Seromed, Berlin). Blood from human volunteers was obtained from the cubital vein.

Culture methods and stimulants used: Lymphocytes were isolated from marmoset or human blood using a modification of the Ficoll density gradient separation (7).

Freshly isolated lymphocytes were suspended in RPMI 1640 culture medium (Seromed, Berlin, with 2 g/liter NaHCO₃, without glutamine). L-glutamine (200 mM, lot # 7F52), and 10% fetal calf serum (Seromed, Berlin, lot # 6SO2) were added together with 100 U penicillin and 100 µg streptomycin (Seromed, Berlin, lot # 8R08). Substances used for stimulation were present in the medium from the beginning of culturing. The cells were incubated in 96-well NUNC plates (Nunc Microwell Plate). Then 200,000 cells were added to 200 µl of the culture medium and they were incubated for periods of up to twelve days at 37°C in a 5% CO₂/95% air atmosphere.

Substances used for stimulation were: recombinant human IL-4 obtained from Dunn Labortechnik (Asbach, FRG; lot # 20506493, from *E. coli*), dexamethasone (Fortecortin®) purchased from Merck, Darmstadt (Ch.-B. 06311814), and the lipopolysaccharide from Sigma (LPS *E. coli*, 0127:B8, lot # L-4516).

Analyses of IgE levels and kits used: IgE was measured in blood plasma as well as in cell-free supernatants after culturing. Plasma was obtained by centrifugation of heparinized blood. Supernatants were obtained by aspiration (with a plastic tipped Gilson pipette) of 170 µl supernatant at the end of the culturing period.

The IgE kits used were: Pharmacia CAP-RAST®FEIA (Pharmacia, Freiburg, FRG; cat.# 52-1937-06), Pharmacia Phadezym IgE Prist® (Pharmacia, Freiburg, FRG; cat.# 10-5589-01), Pharmacia IgE RIAC®

(Pharmacia, Freiburg, FRG; cat.# 52-1921-05), IgE-Immunoenzymometric Assay Kit (Immunotech S.A, Marseille, France; cat.# 0733) and Kallestad Total IgE Microplate (Sanofi Pasteur, Chaska, USA; cat.# 1290).

Cell proliferation: Cell proliferation was measured by incorporation of ^3H -thymidine. After periods of up to twelve days of culturing, 10 μl of ^3H -thymidine containing 1 mCi/ml (75 GBq/mmol, 2 Ci/mmol) were pipetted to each well and culturing was continued for 6 hours. The cells were collected on filter paper (Skatton, Filtermats, lot # 910607) using a cell harvester (Titertek[®], Flow Laboratories), dried and the radioactivity was measured in a liquid-scintillation counter (Tri-Carb[®] Analyser 2200 CA) using 10 ml of Quick-safe[®] (Zinsser Analytik, Frankfurt) scintillation fluid. Evaluated were the stimulation indices (cpm in the presence of stimulants/reference values of the same experiment).

Analyses of surface receptors and antibodies used: Measurements of the surface receptors were performed with the following monoclonal antibodies (mAbs):

anti-CD20 (B₁, H299; lot # 0302 C 294), from Coulter Electronics (Krefeld, FRG), *anti-CD23* (Leu20, EBVCS5; lot # P1104), from Becton Dickinson (Heidelberg, FRG), monoclonal antihuman IgE DE1 epitope (5G11-11-7; lot # 3), and monoclonal antihuman IgE DE2 epitope (E1242-8; lot # 4) both from Bibby Dunn Labortechnik (Asbach, FRG). The cross-reactivity of the monoclonal antibodies directed against surface proteins had previously been determined, no cross-reactivity against specific human IgE could be obtained with either antibody.

For the analysis of the cell surface receptors the cells were removed from the individual wells of the incubation plate, and they were washed twice with PBS (finally with an addition of 0.1% NaN₃). The cell number was measured with an F-800 Microcell counter (Digitana, Hamburg), and the cell count was adjusted to 2 Mio cells/ml.

Identification of the receptors with fluorescence-labeled mAbs was performed at 4°C in the dark with an incubation time of 45 minutes. Subsequently, cells were fixed with 1% paraformaldehyde and thereafter they could be kept in the refrigerator (4°C) for periods of up to two days before analysis with the flow cytometer (FACScan, Becton-Dickinson, Heidelberg). Routine double-labeling of the cells was performed using fluorescein isothiocyanate- (FITC) or phycoerythrin- (PE) conjugated mAbs. Cells were pre-gated in order to avoid the measurement of unlabelled cells, and only vital cells were considered (verified by ethidium bromide - acridine orange vitality testing). Analysis was facilitated by using the Winlist[®] software program (Version 2.0, 1992, 1993, Verity Software, Topsham, Ma.). Statistical analysis was performed with Excel[®] (Microsoft) and Minitab[®] software packages.

Results

Plasma levels of IgE in healthy marmosets. In the course of our studies, we tested Pharmacia IgE RIACT[®] IgE-Immunoenzymometric Assay Kit and Kallestad Total IgE Microplate kits, but no detectable levels of IgE could be found in marmoset blood. This may either be due to a poor cross-reactivity of the anti-human mAbs with the IgE of *Callithrix jacchus*, or too low a sensitivity of the IgE kits used.

Subsequently, we tried to use IgE kits containing antihuman polyclonal antibodies, and this attempt was successful. The Pharmacia CAP-RAST[®]FEIA as well as the Pharmacia Phadezym IgE Prist[®] showed a specific cross-reaction with marmoset IgE. In the following studies the CAP-RAST[®]FEIA was preferred because of the higher precision in the analysis.

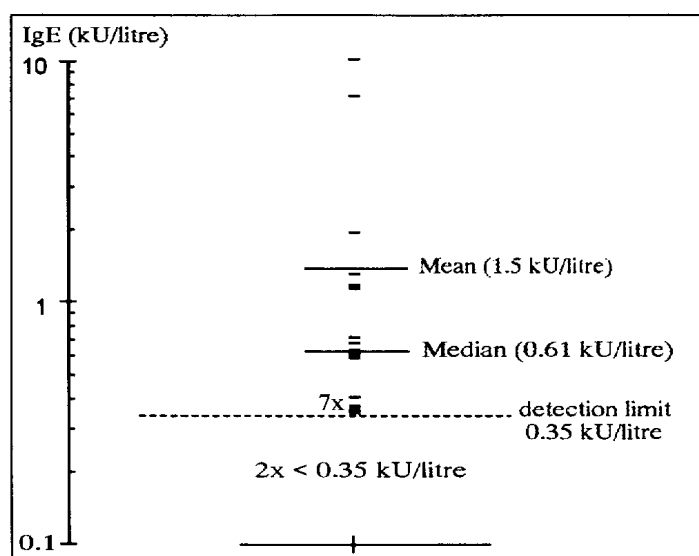


Fig. 1

IgE levels (kU/litre) in the blood plasma of 21 marmosets. Each small bar represents the value of one animal. With the method used, the concentration of two marmosets was below the detection limit of the assay (< 0.35 kU/litre).

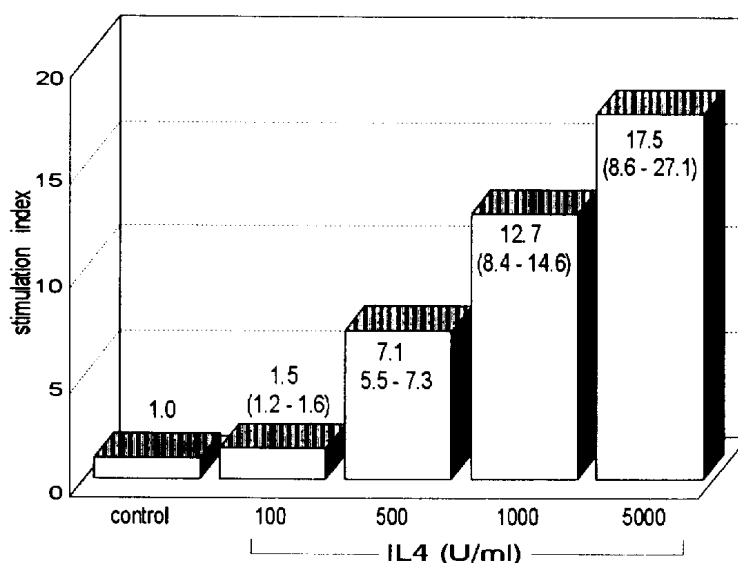


Fig. 2

Stimulation indices of isolated and cultured peripheral lymphocytes from *Callithrix jacchus* after 5 days of stimulation with different concentrations of IL-4. The bars represent median values of at least 4 animals per group. Figures in parentheses indicate the range (minimum and maximum) of the individual values. All measurements were performed in duplicate.

When studying the blood plasma of 21 marmosets, 19 animals showed values above the detection limit of 0.35 kU/litre. However, in the blood of two marmosets, no IgE could be detected with the method used (Figure 1). Even in the animals showing measurable IgE levels, the concentrations varied over a wide range from 10 kU/litre to values close to the detection limit.

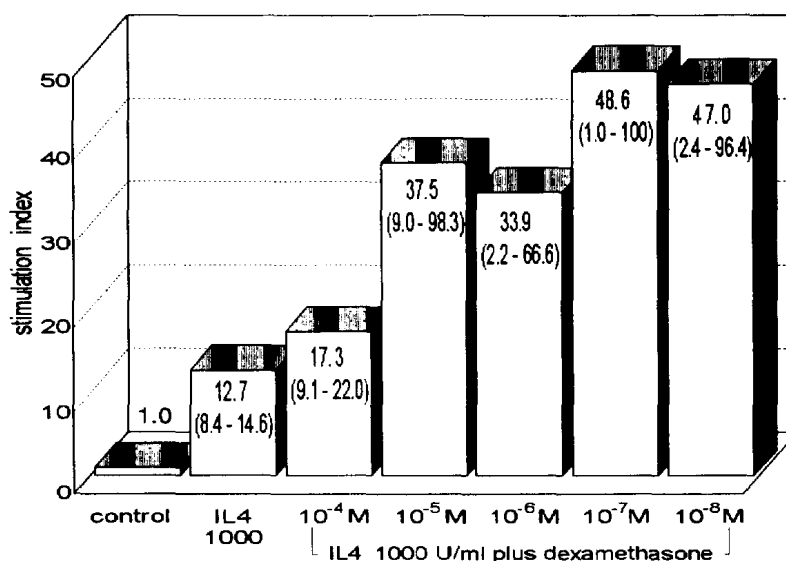


Fig. 3

Stimulation indices (relative proliferation rate compared with controls) of isolated and cultured peripheral lymphocytes from *Callithrix jacchus* after 5 days of stimulation with the constant concentration of 1,000 U IL-4/ml and additionally with different concentrations of dexamethasone. Otherwise, the experimental conditions were the same as indicated for Fig. 2.

Cell proliferation after stimulation with rhIL-4 and dexamethasone. Cell proliferation, as measured by ³H-thymidine incorporation into peripheral blood mononuclear cells, could be enhanced by adding IL-4 to the culture medium (Figure 2). The effect was dose-dependent and was measurable with IL-4 concentrations >100 U/ml. With about 5,000 U/ml, a maximal effect was obtained, the median being about 18-fold above the values obtained in the controls. However, the variability was considerable.

Results from studies performed under identical conditions with human lymphocytes are shown in Tables I and II. Although pointing in the same direction, the results in both primate species were not completely identical. Some stimulation of proliferation can be achieved with human lymphocytes at IL-4 concentrations between 100 and 1,000 U/ml, but about 5,000 U/ml were required for a pronounced effect (Table I). At this high concentration of IL-4, the marmoset lymphocytes clearly responded with a higher rate of proliferation than human lymphocytes.

TABLE I

Stimulation indices after culture of isolated human peripheral blood lymphocytes with different concentrations of IL-4.

Concentration of IL-4	Stimulation index (Median and range)
Controls	1.0
100 U/ml	1.6 (1.6 - 2.0)
500 U/ml	1.6 (1.3 - 1.8)
1,000 U/ml	1.6 (1.1 - 2.0)
5,000 U/ml	3.1 (2.8 - 3.5)

As with marmoset lymphocytes, proliferation could also be induced in human lymphocytes with dexamethasone. However, again a higher concentration (10^{-4} M) was required for human lymphocytes (Table II), while 10^{-8} to 10^{-5} M were already effective in marmoset cells (Figure 3).

TABLE II

Stimulation indices after culture of isolated **human** peripheral blood lymphocytes with IL-4 (1,000 U/ml), and different concentrations of dexamethasone.

Concentration of dexamethasone	Stimulation index (Median and range)
0	1.6 (1.1 - 2.0)
10^{-6} M	1.4 (1.4 - 1.5)
10^{-5} M	1.5 (1.3 - 1.7)
10^{-4} M	2.4 (1.5 - 3.0)

Addition of dexamethasone, together with a sub-optimal concentration of IL-4 (1,000 U/ml), further increased the proliferation rate in PBMC of marmosets (Figure 3). The highest proliferation rates under these experimental conditions were obtained with 10^{-8} or 10^{-7} M dexamethasone, while higher concentrations of the glucocorticoid proved to induce a less pronounced response.

Changes in expression of the CD23 receptor after IL-4 stimulation: Lymphocytes of marmosets could be induced by IL-4 to express the CD23 receptor on B cells (CD20⁺) in culture. This effect was also concentration-dependent, being clearly demonstrable at concentrations of 250 U IL-4/ml or higher. Above 500 U IL-4/ml, a plateau was reached for the median values, and about $20 \pm 10\%$ of the cells were CD20⁺CD23⁺. The variability of the response increased with higher concentrations (Figure 4).

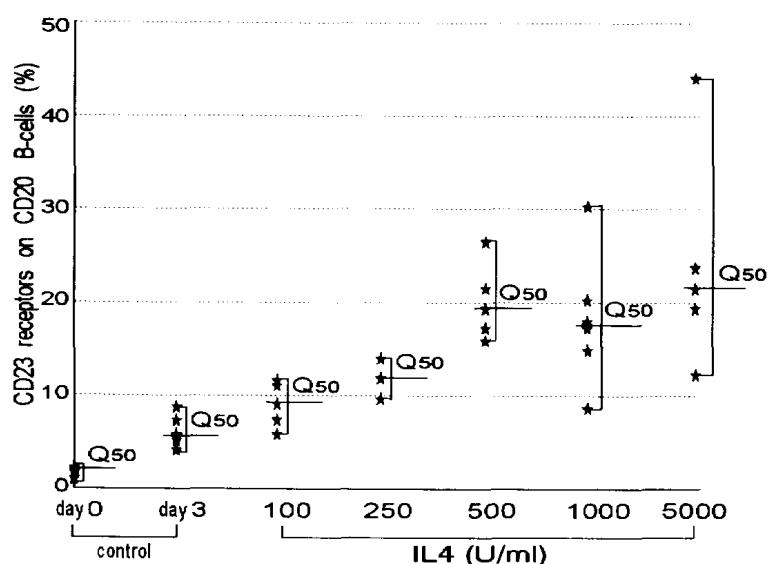


Fig. 4

Percentage of CD23-positive cells within the CD20 B-cell subset of isolated and cultured peripheral lymphocytes from venous blood of *Callithrix jacchus* after 3 days of stimulation with different concentrations of IL-4. Median values (Q50) and the range (minimum and maximum) are shown. A statistically significant, concentration-dependent increase of the CD23-positive B-cells is observed. Concentrations higher than 500 U IL-4/ml do not induce a further increase.

When the same experimental design was used with isolated human lymphocytes, a plateau was already reached with 100 U IL-4/ml, and about 65% of the cells acquired the CD23 receptor (Table III).

IgE production in the presence of IL-4 alone or in combination with dexamethasone or LPS. There was no measurable IgE (i.e. it was < 0.35 kU/liter) in the cell-free supernatant of lymphocyte cultures from 5 different marmosets after 12 days of culturing in the absence of IL-4 or in the presence of <5,000 U IL-4/ml (Table IV). However, with the highest concentration of IL-4 used, IgE was detected at a concentration of 0.8 kU/liter in the culture of one of the five animals.

When IL-4 was combined with either dexamethasone or LPS, a detectable synthesis of IgE was observed in the cultures of all five marmosets, but the concentrations of IL-4 as well as of dexamethasone required to induce a measurable response varied considerably for the lymphocytes of the different animals. With the lymphocytes of one of the marmosets IgE was formed occasionally in the presence of IL-4 at a concentration of 100 or 500 U/ml, but this effect was not reproduced consistently. While 1,000 U/ml IL-4 alone were insufficient to induce the formation of IgE, the combination with 10^{-6} M to 10^{-4} M dexamethasone was able to trigger the formation of this immunoglobulin with the cells from four of the five marmosets tested. An IgE concentration exceeding 1 kU/l was seen with 5,000 U IL-4/ml and 10^{-6} to 10^{-7} M dexamethasone with the lymphocytes from one of three marmosets. The other animals showed values barely above the detection limit. Interestingly, the highest concentration of dexamethasone, namely 10^{-4} M, was not more effective than 10^{-5} or 10^{-6} M, and frequently the highest dose was apparently rather inhibitory. A pronounced response was seen in all three marmosets tested in the presence of 50 µg lipopolysaccharide/liter and 1,000 U IL-4/liter (2.4 - 4.8 kU/liter).

TABLE III
Percent of B cells with the CD23 receptor after culture of isolated human peripheral blood lymphocytes with different concentrations of IL-4

Day of culture	Concentration of IL-4	Percent of CD23-positive B cells
Day 0	0	40.0 (32.1 - 53.2)
Day 3	0	7.4 (3.2 - 12.1)
Day 3	5 U/ml	8.4 (2.1 - 12.1)
Day 3	10 U/ml	16.3 (12.1 - 21.0)
Day 3	50 U/ml	45.3 (133.7 - 57.9)
Day 3	100 U/ml	64.7 (52.6 - 70.5)
Day 3	500 U/ml	66.8 (52.8 - 73.7)
Day 3	1,000 U/ml	64.9 (51.6 - 71.4)
Day 3	5,000 U/ml	67.8 (54.4 - 71.6)

Discussion

There is little information available on the immunoglobulin system of the whole group of New World monkeys. Cross-reactivity of a polyclonal antibody against a surface antigen of a T cell line with the heavy chain of human IgG and with the heavy chain of a serum-Ig of *Saguinus oedipus* (cotton-topped marmoset) has been reported (8). To our knowledge, no data have been published on the IgE system of *Callithrix jacchus*. Stimulation of the proliferation of marmoset B cells by human IL-4 has been reported earlier (9), but no analysis of IgE synthesis was performed. With respect to other non-human primates, some evidence of a functional cross-reactivity between human IgE and that of an Old World monkey, in this case Rhesus, was published (10).

It is well-known that marmosets living in the wild are heavily infected with worms and with other parasites (11). Therefore, it would be understandable that these species developed an IgE system similar to that of humans. The data presented here provide evidence that an IgE system at least similar to the human one exists in *Callithrix jacchus*, although the plasma IgE levels found in our studies are clearly lower than in humans. Since there are no worm infections whatsoever in our marmoset colony, a potential IgE stimulus may be missing. It will be interesting to test whether exposure to parasite antigens will greatly stimulate the IgE system in the marmoset. This would provide an excellent tool for evaluating possible substance-related effects on such an allergy model. Similarities in some immune responses to dexamethasone between marmosets and humans have been demonstrated with respect to the increase of a macrophage subtype (RM 3/1⁺) *in vivo* (12).

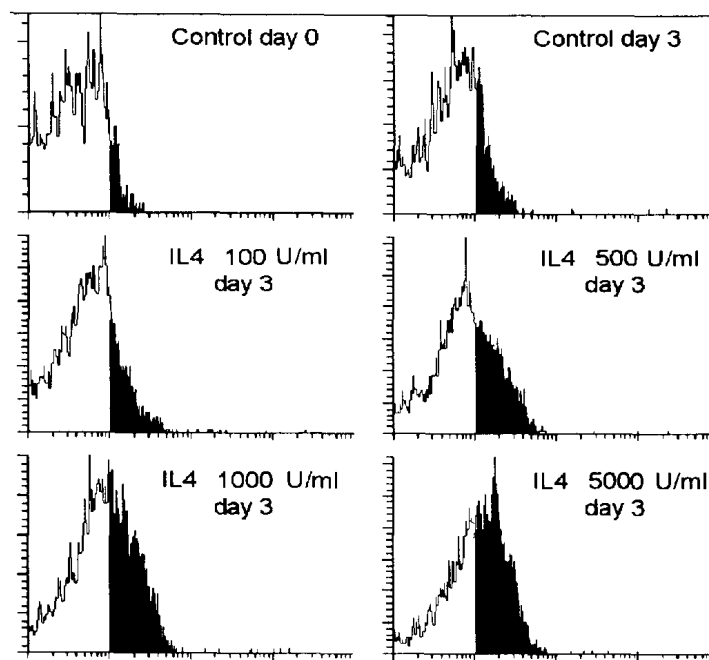


Fig. 5

Original FACScan histograms. Lymphocytes from marmoset blood were pre-gated for the CD20-positive fraction. Example of the increase of the CD23 receptor on CD20-positive B cells after stimulation with various concentrations of IL-4 *in vitro*. The number of cells is plotted against the fluorescence intensity (mV).

In addition to the numerical increase of CD23-positive cells, a slight increase of the receptor density of some cells can be observed.

CD23, the low-affinity IgE receptor FcεRII, is induced on human B cells after stimulation with IL-4 (13). The significance of this receptor for the induction and regulation of the IgE system is well-established (14,15,16). Apparently, the CD23 receptor is an important and specific mediator and marker for the development of B cells to IgE-producing plasma cells. The fact that we find similarities in the response of cells from humans and marmosets suggests that this non-human primate and humans use similar means of regulating and expressing the IgE system.

Comparison of the effects of IL-4, glucocorticoids or lipopolysaccharide on IgE synthesis and CD23 expression in various species *in vitro* is difficult because the experimental conditions differ considerably from investigator to investigator.

Apparently, IL-4 alone is only capable of inducing slight changes in IgE synthesis. This holds true (17,18) for peripheral blood lymphocytes of normal human donors, and this is the result of our studies with marmosets. However, it is interesting that lymphocytes of patients with atopic dermatitis were reported to be more susceptible to the induction of IgE synthesis by IL-4 *in vitro* (17,18).

On the other hand, CD23 expression is inducible by IL-4 alone in lymphocytes of healthy human donors at concentrations of 10 U IL-4/ml (17,19). Similar results are observed with lymphocytes of marmosets, but higher concentrations of IL-4 may be required (500 U/ml) for an optimal stimulation.

TABLE IV
Concentration of IgE in the supernatants of cultures of venous blood lymphocytes from
Callithrix jacchus after stimulation with various concentrations of
IL-4, dexamethasone and lipopolysaccharide (LPS).
Data are given as kU IgE/liter. * : < 0.35 kU/liter open spaces: not measured

IL-4 (U/ml)	Dexamethasone	Marmoset				
		# 1	# 2	# 3	# 4	# 5
Controls		*	*	*	*	*
100		*	*	*	*	*
1,000		*	*	*	*	*
5,000		*	*	*	*	0.76
100	10 ⁻⁷ M	*			*	*
100	10 ⁻⁶ M	*			0.68	*
100	10 ⁻⁵ M	*			0.43	*
100	10 ⁻⁴ M	*			*	*
500	10 ⁻⁷ M	*			*	*
500	10 ⁻⁶ M	*			*	*
500	10 ⁻⁵ M	*			0.48	*
500	10 ⁻⁴ M	*			*	*
1,000	10 ⁻⁷ M	*	*	*	*	*
1,000	10 ⁻⁶ M	*	0.41	*	*	*
1,000	10 ⁻⁵ M	0.37	0.63	*	*	*
1,000	10 ⁻⁴ M	0.38		0.44	0.76	*
5,000	10 ⁻⁷ M	*			*	1.28
5,000	10 ⁻⁶ M	*			*	2.66
5,000	10 ⁻⁵ M	*	0.57	0.54	*	
5,000	10 ⁻⁴ M	0.38			*	0.43
1,000	50 µg/ml LPS	4.83			2.39	2.50

Simultaneous stimulation by IL-4 (10 U/ml) and prednisolone (10⁻⁷ M) was shown to greatly enhance IgE synthesis of human peripheral blood mononuclear cells from healthy donors after 14 days of culturing, but only a very slight effect was seen after 7 days. However, mononuclear cells from different donors exhibited a considerable variability in the response to prednisolone alone (10⁻⁷ M) and also to the combination of prednisolone plus IL-4 (10 U/ml), the IgE formation ranging from 0.8 to 15.3 ng IgE/ml in the case of prednisolone alone, and from 1.5 to 142 ng IgE/ml for the co-stimulation. The ratio of the stimulatory effect be-

tween prednisolone alone and prednisolone plus IL-4 ranged from 1.3 to 20 (18). While the combination of prednisolone plus IL-4 caused a strong stimulation of the IgE synthesis, prednisolone combined with IL-2 produced no altered response in the lymphocytes of the normal donors, and rather a decreased response when PBL from donors with spontaneous IgE synthesis were used (20).

In contrast, the expression of CD23 on human lymphocytes was slightly reduced in the presence of 10^{-7} to 10^{-5} M prednisolone, and no impressive effect was seen with the combination of prednisolone and IL-4. However, the release of sCD23 was greatly enhanced in the presence of this combination (20). This co-stimulation with IL-4 plus glucocorticoids was also found to be capable of inducing IgE synthesis in lymphocytes of marmosets, but again, higher concentrations were required. As found for humans, the individual variability was also considerable in *Callithrix*, and the cells of not all marmosets responded under the same experimental conditions.

An important role of cell-cell interactions between B cells, T cells and monocytes in the induction of CD23 expression was suggested (21). Additionally, the IL-4-induced IgE synthesis is enhanced in the presence of monocytes. Depletion of monocytes from a preparation of mononuclear cells strongly alters the IgE secretion in the cultures after prednisolone stimulation. However, glucocorticoid-induced IgE synthesis is not dependent on IL-6. Monocyte contact with B cells was found to be sufficient to enhance IgE synthesis, and LFA-1 (CD11a) and ICAM-1 (CD54) are suggested to be involved in mediating the cellular contact signal required for glucocorticoid-induced IgE synthesis (18).

Data on possible effects of antigens on the IgE system reveal a rather complex situation. Reports on effects observed in different species are rather controversial, often due to incomparable experimental conditions. An IgE-response to lipopolysaccharide is apparently possible in mice (22,23). Furthermore, IgE synthesis in isolated B cells from *Nippostrongylus brasiliensis*-reinfected mice was reported to be moderately stimulated by the antigen, but very strongly stimulated by IL-4, while the simultaneous action of the two components induced a weaker response than IL-4 alone (24).

Rather conflicting results were also reported from studies with human lymphocytes *in vitro*. This again may be due to different experimental approaches. It has been claimed that an IgE response by lipopolysaccharide cannot be induced in humans (16,25). However, other reports indicate a much more complex situation: as might be expected, IL-4 was found to increase the CD23 expression on peripheral blood lymphocytes of normal donors as well as of patients with atopic dermatitis *in vitro*, but IgE synthesis was only increased in cells from patients with atopic dermatitis. Co-stimulation of cells *in vitro* with cell wall products of *Staphylococcus aureus* (teichoic acid of peptidoglycan) plus IL-4 led to a pronounced increase in IgE synthesis in lymphocytes of patients with atopic dermatitis (17), and the authors suggested that this modulation of humoral immunity may be responsible for allergic skin reactions in such patients. On the other hand, co-stimulation with teichoic acid or enterotoxin B caused a decrease of the CD23 expression in lymphocytes of normal donors, but an increase in the CD23 expression in cells of patients with atopic dermatitis.

Our results show that peripheral lymphocytes from marmosets also can be induced to produce IgE *in vitro* in a very similar manner to equivalent experimental set-ups using human lymphocytes (26,27). In general, however, the concentrations of IgE obtainable in comparable cultures are lower with cells from marmosets than with human cells. This may be due to the initially lower plasma concentrations of IgE in marmoset blood as well as to the fact that much fewer marmoset lymphocytes show low affinity IgE receptors after stimulation with IL-4. This leads to the assumption that generally fewer B-cells can be induced to produce IgE under these culture conditions. A *quantitative* assessment of the IgE synthesis can only be achieved with difficulty, since the values obtained through stimulation with rh-IL-4 alone were too variable and too low to be accurately determined. The *qualitative* statement, however, that marmoset lymphocytes can be induced to produce measurable quantities of IgE through stimulation with combinations of rh-IL-4 and dexamethasone as well as rh-IL-4 and lipopolysaccharide holds true, keeping in mind that the latter stimulation pathway is apparently ineffective in human cultures with mononuclear cells. Such differences must be known and taken into account when using *Callithrix jacchus* as a model for elucidating possible effects with relevance for man.

The results of these studies and the similarity with the human response encourage the development of an allergy model system with the marmoset, monitoring the response of the IgE system to a challenge by parasite antigens. Such a possibility to investigate the IgE system under different conditions may be helpful for the exploration of new therapeutic strategies in atopic diseases.

Acknowledgments

We thank Prof. Dr. med. Ulrich Wahn, Children's Hospital, Berlin, Prof. Dr. med. Wolfgang König, Bochum, for helpful discussions, and Doris Webb for the help in preparing the manuscript. The expert technical assistance of Ursula Jacob-Müller is gratefully acknowledged.

References

1. R. NEUBERT, A.C. NOGUEIRA, H. HELGE, R. STAHLMANN and D. NEUBERT, Risk Assessment of Prenatally-induced Adverse Health Effects, D. Neubert, R.J. Kavlock, H.-J. Merker (Eds), 313-346, Springer Verlag, Berlin (1992).
2. D. NEUBERT, S. KLUG, G. GOLOR, R. NEUBERT and A. FELIES, Risk Assessment of Prenatally-induced Adverse Health Effects, D. Neubert, R.J. Kavlock, H.-J. Merker (Eds), 347-376, Springer Verlag, Berlin (1992).
3. R. NEUBERT, H. HELGE and D. NEUBERT, Immunotoxicology, M.P. Holsapple, R.J. Smialowicz (Eds), CRC Press, Boca Raton, NY, London, Tokyo, pp 63-98 (1995).
4. R. NEUBERT, R. STAHLMANN, M. KORTE, H. Van LOVEREN, J.G. VOS, G. GOLOR, J.R. WEBB, H. HELGE and D. NEUBERT, *Ann. N.Y. Acad. Sci.* **685** 662-686 (1993).
5. R. NEUBERT, M. FOERSTER, A.C. NOGUEIRA and H. HELGE, *Life Sciences* **58** 317-324 (1995/96).
6. H.H. JABARA, R. LOH, N. RAMESH, D. VERCELLI and R.C. GEHA, *J. Immunol.* **151** 4528-4533 (1993).
7. A. BÖYUM, *Scand. J. Clin. Invest.* **21** (Suppl. 97) 77-89 (1966).
8. M.T. SPEIDEL and J. MARCHALONIS, *Exp. Clin. Immunogenet.* **6** 245-50 (1989).
9. D.J. QUINT, S.P. BUCKHAM, E.J. BOLTON, R. SOLARI, B.R. CHAMPION and E.D. ZANDERS, *Immunology* **69** 616-621 (1990).
10. K. ISHIZAKA, and T. ISHIZAKA, *J. Immunol.* **100** 554-562 (1968).
11. S. POTKAY, *J. Med. Primatol.* **21** 189-236 (1992).
12. G. ZWADLO-KLARWASSER, R. NEUBERT, R. STAHLMANN and W. SCHMUTZLER, *Int. Arch. Allergy Immunol.* **97** 178-80 (1992).
13. T. DEFRANCE, J.P. AUBRY, F. ROUSSET, B. VANBERVLIET, J.Y. BONNEFOY, N. ARAI, Y. TAKEBE, T. YOKOTA, F. LEE, K. ARAI, J. DEVREIS, and J. BANCHEREAU, *J. Exp. Med.* **165** 1459-1467 (1987).
14. W. KÖNIG, J. BUJANOWSKI-WEBER, A. FISCHER, J. FRÄNKEN, I. KNÖLLER, K. NEUBERT and U. STEPHAN, *Allergologie* **14** 1-23 (1991).
15. M. SARFATI and G. DELESPESE, *J. Immunol.* **141** 2195-2199 (1988).
16. D. VERCELLI and R. GEHA, *J. Allergy Clin. Immunol.* **88** 285-295 (1991).
17. K. NEUBERT and W. KÖNIG, *Immunology* **75** 23-28 (1992).
18. A. FISCHER and W. KÖNIG, *Immunology* **74** 228-233 (1991).
19. A. FISCHER, T. PFEIL and W. KÖNIG, *Int. Arch. Allergy Appl. Immunol.* **92** 334-342 (1990).
20. A. FISCHER and W. KÖNIG, *Immunology* **71** 473-479 (1990).
21. K. KICZA, A. FISCHER, T. PFEIL, J. BUJANOWSKI-WEBER and W. KÖNIG, *Immunology* **68** 532-539 (1989).
22. R. COFFMAN and J. CARTY, *J. Immunol.* **136** 949 (1986).

23. R. COFFMAN, J. OHARA, M. BOND, J. CARTY, A. ZLOTNIK and W. PAUL, *J. Immunol.* 136 4538-4541 (1986).
24. U. STEPHAN and W. KÖNIG, *Int. Arch. Allergy Immunol.* 98 299-307 (1992).
25. J. SPLAWSKI, D. JELINEK and P. LIPSKY, *J. Immunol.* 142 1569-1575 (1989).
26. M. SARFATI, H. LUO and G. DELESPESE, *J. Exp. Med.* 170 1775-80 (1989).
27. H. JABARA, D. AHERN, D. VERCELLI and R. GEHA, *J. Immunol.* 147 1557-60 (1991).