ROLE OF INTERLEUKIN-4 IN THE INDUCTION OF HUMAN IgE SYNTHESIS AND ITS SUPPRESSION BY INTERFERON-γ•

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Studies on *in vitro* synthesis of human IgE have been limited, for a long time interval, to the evaluation of the spontaneous IgE production by atopic B cells[8]. However, some laboratories have recently been successful in demonstrating the possibility of inducing an *in vitro* polyclonal IgE response under particular experimental conditions using filarial parasite-specific T cell lines[6], as well as selected alloreactive[3,7,12] or autoreactive[4] helper T cell clones (TCC). More recently, we found that several TCC established from tonsillar or peripheral blood (PB) T lymphocyte suspensions of nonallergic individuals by stimulation of single T cells with phytohemagglutinin (PHA) provided helper function for IgE synthesis in B cells from both allergic and nonallergic donors, regardless of allo- or autoantigen recognition or specificity for peculiar antigens[2,10].

The present study was designed to examine whether supernatants (SN) derived from these clones were able to provide IgE helper function in the absence of the T cells themselves. We have established that SN from TCC active on IgE synthesis could induce IgE production in B cells. Such an activity was related to the presence of interleukin-4 (IL-4) in SN and was inhibited by interferon-γ (IFN-γ).

MATERIALS AND METHODS

Reagents - Phytohemagglutinin M was purchased from GIBCO (Grand Island, N.Y.); interleukin-2 (IL-2) and IFN-γ, obtained by the recombinant DNA

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technology (rIL-2 and rIFN-γ), were kindly provided by Biogen (Geneva); anti-CD3 (OKT3), anti-CD4 (OKT4) and anti-CD8 (OKT8) monoclonal antibodies (MoAbs) were purchased from Ortho Pharmaceutical Co. (Raritan, N.J.); the B1 MoAb (anti-B lymphocyte) was purchased from Kontron (Zurich, Switzerland). The production and characterization of anti-human ε-chain MoAb (E-45) has been previously described in detail[8]. A rabbit antibody specific for the human ε-chain was prepared in our laboratory[8]. Affinity-purified $F(ab')_2$ fragments of rabbit antibodies against mouse immunoglobulins were prepared and conjugated with fluorescein-isothiocyanate, as previously reported[11].

Preparation of TCC - TCC were established according to the technique described by MORETTA et al.[5], as detailed elsewhere[2,10].

Preparation of TCC SN - Viable T blasts from 15 selected TCC (5 from tonsil G, 5 from PB H and 5 from PB J) were recovered on Ficoll-Hypaque gradient, extensively washed, resuspended at 10^6/ml (if not otherwise stated) of complete medium in the absence or presence of 1% (v/v) PHA, incubated at 37 °C for 24h and centrifuged at 400 *g*; culture SN were then collected. SN were filtered through a 0.22-μ filter and stored in aliquots at −70 °C until used.

B cell donors - Five adult grass pollen- and/or mite-sensitive subjects with allergic rhinitis and/or extrinsic asthma and serum IgE levels > 300 IU/ml were used as B cell donors. These patients were selected because in previous assays their B cells usually did not show spontaneous IgE synthesis or synthesized very small amounts of IgE *in vitro*.

TCC SN added to B cells	B cell donor 1*		B cell donor 2*	
	IgE (ng/ml)	IgG (μg/ml)	IgE (ng/ml)	IgG (μg/ml)
none	0.4**	0.1	0.3	0.2
G.9	6.4	8.3	2.0	3.3
G.11	7.1	9.4	1.8	5.8
G.20	3.2	6.9	2.7	4.8
G.26	4.0	0.8	2.4	0.7
H.3	7.4	10.4	3.9	5.3
H.12	6.0	7.9	3.1	6.8
H.31	3.9	1.2	4.2	1.0
H.40	2.8	5.1	1.7	4.2
J.16	2.3	3.4	2.8	3.8
J.29	4.7	0.5	3.5	0.6
G.13	0.4	3.2	0.3	1.9
H.22	0.5	1.7	0.4	2.2
J.1	0.3	4.3	0.3	3.6
J.24	0.3	1.9	0.4	0.5
J.37	0.4	8.5	0.3	6.4

* B cells (4×10^5/ml) from 2 atopic donors were cultured in the medium alone or in the presence of TCC SN (50% final concentration). After 10 days, culture supernatants were assayed for IgE and IgG content.
** Values represent the mean of triplicate determinations.

Tab. 1 - *In vitro* IgE synthesis induced by SN of PHA-stimulated TCC.

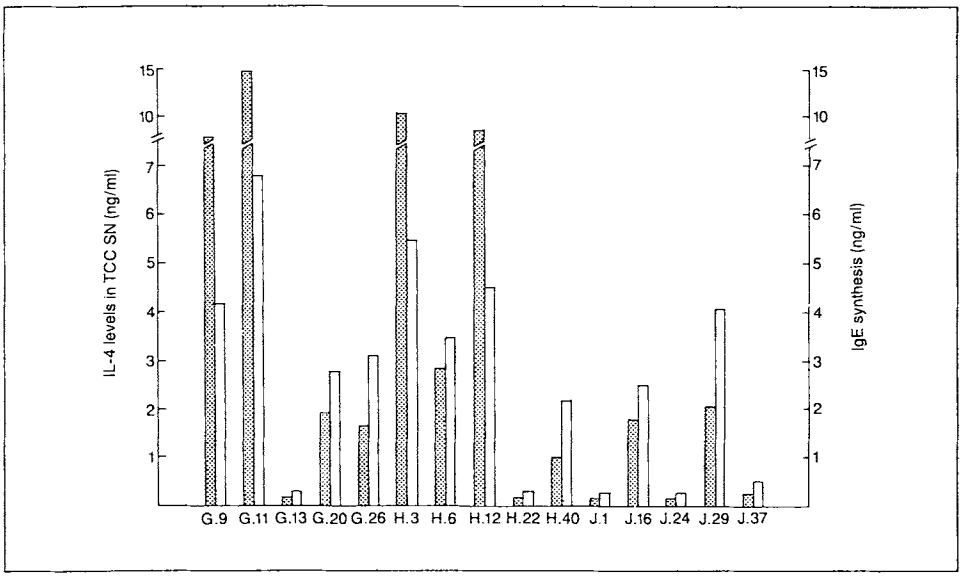

Fig. 1 - Ability of TCC SN to induce *in vitro* IgE synthesis (white columns) and their IL-4 content (dotted columns).

Preparation of B cells - B cell-enriched suspensions were prepared as described in detail elsewhere[2,10] and usually consisted of 60-80% B cells and less than 1% T cells, as judged by indirect immunofluorescence with B1 and OKT3 MoAb, respectively. They will be simply referred to as B lymphocytes.

Cell culture - The cell culture system used for inducing Ig synthesis was performed in duplicate in 12 × 75 mm plastic tubes (Falcon Plastic, Oxnard, CA) using complete medium in a humidified atmosphere of 5% CO in air. Each tube contained 4×10^5 B cells and the corresponding SN preparations in 1-ml final volume. After 10 days, culture supernatants were collected and assayed for their IgE and IgG content.

Measurement of IgE and IgG - The procedure for the measurement of IgE has been described in detail elsewhere[2,10]. The lower sensitivity value of the test was 0.3 ng/ml and the mean intra-assay coefficient of variation was 17%. *De novo* or net IgE synthesis was calculated by subtracting the IgE values obtained in parallel cultures containing 100 μg/ml cycloheximide from IgE values found in SN of untreated cultures, as previously reported[8].

The radioimmunoassay used for detecting IgG in culture SN has also been described previously in detail[9].

Assessment of IL-4 in TCC SN - IL-4 levels were measured in TCC SN by an immunoenzymatic assay (courtesy of Drs J. Bancherau, J. de Vries and I. Chretien, UNICET, Dardilly).

RESULTS AND DISCUSSION

SN of 15 $CD4^+$ TCC derived from tonsil or PB of 3 different normal individuals were examined for their ability to induce IgE production in B cells

TCC SN	IFN-γ (IU/ml) added*			
	–	10	50	250
–	0.3	0.2	0.3	0.3
G.26	3.9	3.8	2.8	1.9
G.11	5.8	6.2	3.0	2.0
J.16	3.2	2.5	1.8	1.2
J.29	2.9	2.4	0.9	0.6

* B cells (4×10^5/ml) from one atopic donor were incubated for 10 days with SN of four different TCC in the absence or presence of different concentrations of IFN-γ.

Tab. 2 - Inhibitory effect of IFN-γ on the IgE synthesis induced by TCC SN.

from 2 atopic donors. To this end, B cells were incubated for 10 days with SN derived from clonal T cells stimulated with 1% (v/v) PHA. IgE and IgG released in B cell culture supernatants were then measured. Under the above experimental conditions, the incubation of B cells with supernatants derived from 10 PHA-stimulated TCC, previously shown to be able to induce IgE synthesis in target B cells, resulted in a substantial IgE production *in vitro*, whereas SN from 5 TCC inactive on IgE synthesis were unable to induce IgE synthesis in B cells from both atopic donors. In contrast, SN from all the 15 TCC induced production of substantial IgG amounts in target B cells (tab. 1).

It was recently shown that murine IL-4 is able to induce IgE production in activated B cells and that its activity is inhibited by IFN-γ[1]. To establish whether IgE helper activity of our TCC SN was related to the presence of IL-4 as well, IL-4 levels were measured in either active or inactive SN by an enzyme-linked immunosorbent assay (ELISA). The results of these experiments are summarized in fig. 1. All 10 SN active on IgE synthesis had detectable or even elevated IL-4 concentrations, whereas IL-4 was virtually undetectable in SN unable to induce IgE synthesis.

The effect of different concentrations of IFN-γ on IgE synthesis induced by active TCC SN was also evaluated. The addition of IFN-γ to B cell cultures induced a dose-dependent inhibition of the IgE synthesis stimulated by TCC SN (tab. 2).

Taken together, these data suggest that IL-4 probably plays an important role in the induction of IgE synthesis by TCC SN and that its IgE helper activity is modulated by IFN-γ. Experiments are now in progress to establish whether IL-4 is acting alone or in concert with other lymphokines in the induction of IgE synthesis.

SUMMARY

Supernatants (SN) from 10 phytohemagglutinin (PHA)-stimulated human T cell clones (TCC), selected for their helper function on IgE synthesis, were found to provide IgE helper activity in atopic B cells showing low or undetectable spontaneous *in vitro* IgE synthesis. In contrast, SN from 5 PHA-stimulated TCC unable to provide helper function for IgE synthesis consistently failed to elicit IgE production. SN active on IgE synthesis contained high concentrations of interleukin-4 (IL-4), whereas inactive SN did not contain detectable amounts of IL-4. Moreover,

the IgE helper activity of TCC SN was strongly inhibited by the addition of interferon-γ (IFN-γ) to B cell cultures. These data suggest that IL-4 may play a role in the induction of *in vitro* human IgE synthesis, whereas IFN-γ displays an inhibitory effect.

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