

IgE-producing hybridomas established after B-cell culture in the CD40 system

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1. Summary

While total IgE synthesis can be easily induced in human PBL or B cells by different stimuli, no systems are known for the induction of allergen-specific IgE in vitro. In this study we investigated whether a specific Ig response could be induced using the CD40 culture system with the final intention to generate B-cell hybridomas secreting IgE of defined specificity. B cells derived from immunized donors normally give rise to many specific hybridomas after cell fusion. However, if cultured in the CD40 system and then immortalized and screened for anti-tetanus specificity, no tetanus-specific clones were found but a large number of IgE-secreting hybridomas had been generated. Also allergen-specific B cells could not be expanded in the CD40 system but long-term cultures yielded again B cells that were efficiently immortalized by cell fusion resulting in stable IgE-secreting hybridomas but of undefined specificity. One of these IgE-producing clones was further characterized and had an IgE production rate of 4.5 $\mu\text{g}/10^6$ cells/24 h. This paper provides two findings. (1) Our cell lines represent a valuable new source of human IgE. (2) Most importantly, our data indicate that the CD40 system is not suitable to expand specific B cells, suggesting that other systems have to be developed for the induction of a significant antigen-specific Ig response.

2. Introduction

It is well established that IL-4 plays a central role in IgE synthesis by inducing isotype switch in B cells, an

effect which is antagonized by IFN γ [1,2]. More recently IL-13 has been described as a second cytokine capable of induction of IgE synthesis [3]. For efficient B-cell activation, however, a second signal seems to be required. It has been shown that this second signal can be provided by activated T cells [4], hydrocortisone [5] or EBV infection [6]. Numerous reports demonstrate that B cells activated either with anti-CD40 antibody [7–10] or the CD40 ligand present on activated T cells [11–13] produce large amounts of IgE in the presence of IL-4. For long-term growth of B cells, Banchereau et al. reported a system in which B cells are expanded in the presence of IL-4 on a layer of irradiated Ltk-fibroblasts transfected with Fc γ RII (CD32) that present anti-CD40 antibody [14,15]. In this system, referred to as the CD40 system, B cells can be clonally expanded with high efficiency [14].

In contrast to this well established in vitro IgE synthesis little is known about the specific IgE response in vitro [16–18]. Furthermore, for in vitro study of immediate-type reactions human monoclonal IgE of defined specificity would be of great help. There are at present very few sources of IgE. The most common is the myeloma cell line U266 [19] which was originally derived from a patient suffering from myelomatosis [20]. To circumvent the lack of specificity, cell lines secreting chimeric antibodies have been established, in which variable regions of mouse monoclonal antibodies have been combined with the human C ϵ chain [21,22].

We wanted to use the CD40 system for the clonal expansion of antigen-specific human B cells with the final intention of generating specific IgE-secreting hybridomas. First we studied tetanus toxoid (TTd)-specific B cells as a model because TTd-specific hybridomas can be easily established using a wide range of cellular sources, culture and immortalization systems [36–38]. If cells from immunized donors cultured in the CD40 system were immortalized a large number of IgE-pro-

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ducing hybridomas were established but no TTd-specific clones. Using cells from allergic donors it was again possible to establish IgE-producing clones but none of defined specificity. A recent report demonstrated that in the CD40 system preferentially naive B cells (IgD⁺) were expanded and mature B cells were overgrown and lost if sIgD⁺ cells were present [23]. Thus the CD40 system may not be suitable to expand antigen-specific B cells but provides a powerful tool for the generation of IgE-producing hybridomas.

3. Materials and Methods

3.1. Media and reagents

PBL or purified B cells were cultured in complete IMDM (Iscove's modified Dulbecco's medium) as described elsewhere [24]. U266 cells were cultured in RPMI-1640 supplemented with 10% fetal calf serum (FCS), antibiotics and 2 mM L-glutamine. RPMI-1640 for growth of Ltk-fibroblasts was additionally supplemented with HAT and with geneticine for CHO cells (Gibco, 066-1811, Life Technologies, Basel, Switzerland), respectively.

Anti-CD40 antibody G28-5 from E.A. Clark and recombinant human IL-4 were provided by Sandoz Forschungsinstitut (Vienna, Austria) and used at 100 ng/ml and 50 U/ml, respectively. For culture in the CD40 system, anti-CD40 antibody was used at 500 ng/ml. Purified allergens were obtained from Gerim-mun (Fribourg, Switzerland). Anti-IgE monoclonal antibodies BSW-17 and Le-27 were generated in our laboratory and described elsewhere [25]. IgE ND was purified from cell supernatants of U266, PS was a gift of K. Ishizaka (La Jolla Institute for Allergy and Immunology, CA).

3.2. Cell lines

Ltk-fibroblast cell line transfected with CD32 was obtained from ATCC [26]. CHO cells transfected with Fc ϵ RI was a kind gift of J.-P. Kinet (National Institute of Allergy and Infectious Disease, NIH, Rockville, MD). The heteromyeloma cell line LA55 was used as fusion partner as described previously [27].

3.3. Cell preparation and culture conditions

Mononuclear cells from normal healthy volunteers were isolated from heparinized blood and purified by density centrifugation on Ficoll paque (Nycomed Pharma, Oslo, Norway). The interface fraction was collected and washed 3 times with HBSS. PBL were

grown in TC24 plates (Falcon 3047, Becton Dickinson, Heidelberg, Germany) at a density of 1.5×10^6 cells/ml.

B cells were purified from PBL using a MACS apparatus (Miltenyi, Biotec, Bergisch-Gladbach, Germany) as described [28] by positive selection with anti-CD19 beads. For allergen-specific cell selection, allergens were biotin-labeled using a standard procedure. PBL were incubated for 30 min at 4°C with biotin-labeled allergen and subsequently enriched by positive selection with streptavidin beads on the MACS.

Purified B cells were cultured in TC 96 flat-bottom wells (Falcon 3072) on a layer of 2.5×10^3 irradiated (40 Gy) Ltk-fibroblasts in the presence of IL-4 and 500 ng/ml anti-CD40 antibody (CD40 system).

3.4. Fusion protocol, screening, cloning

Cell fusion, screening and cloning were essentially carried out as described previously in detail [27]. Briefly, PBL or purified B cells were mixed in a 1:1 ratio with LA55 cells. Fusion was carried out in the presence of 40% (w/v) PEG and 5% (v/v) DMSO. Fused cells were then seeded in 96-well flat-bottom plates at a density of 10^4 – 10^5 cells/well in conditioned medium. After 15–18 days culture supernatants were screened for the presence of antigen-specificity. Wells with high IgE production were subsequently cloned at least twice by limiting dilution (10 and 1 cell(s)/well) in round-bottom plates.

3.5. Measurement of IgE, specific IgE and TTd-specific IgG

IgE determination was carried out as previously described with a tandem of monoclonal anti-IgE antibodies recognizing different epitopes, Le-27 and BSW-17 [25].

Specific IgE was detected using a modified RAST. Flexible polystyrene plates were coated with 100 μ l of allergen (15 μ g/ml) in 0.05 M NaHCO₃/Na₂CO₃ overnight at 4°C in a humid atmosphere. Coating solution was discarded and plates blocked with 200 μ l of a 1.5 mg/ml Casein-solution in PBS. Cell supernatants (or sera from RAST-positive patients as positive controls) were added overnight at room temperature in a humid atmosphere. Plates were then washed 3 times with 0.05% Tween in 0.9% NaCl. Next I¹²⁵-labeled BSW17 was added for 6 h, plates washed 4 times with Tween as above, dried, cut into single wells and cpm measured in a gamma-counter (Gammamatic, Kontron Instrumente, Zürich, Switzerland). Anti-TTd antibodies were detected by ELISA as described previously [30]. Alternatively TTd-specific IgG was measured as spe-

cific IgE (as described above) using flexible polystyrene plates coated with TTd (10 mg/ml) and I^{125} -labeled monoclonal anti-IgG antibody BS-17 (Biotest, Frankfurt, Germany) for development.

3.6. SDS-PAGE and Western blot

SDS-PAGE and Western blots were performed as described earlier [31]. Briefly, a gel with separating zones of 10% (w/v) acrylamid/bisacrylamid (40:1) was run in a Mini-Protean II apparatus (Bio-Rad, Richmond, CA). Running buffer consisted of 0.1% (w/v) sodium dodecyl sulfate (Fluka, Buchs, Switzerland) in 24.8 mM Tris (Merck, Darmstadt, Germany), 191.8 mM glycine (Merck), pH 8.2 and 5% (v/v) 2-mercaptoethanol (Merck). Samples containing 15.6 mM Tris-HCl, pH 6.8, 0.5% (w/v) SDS, 3.7% (v/v) glycerol (Biofinex, Praroman, Switzerland), 0.0005% (w/v) bromphenol blue were heated to 95°C for 5 min and 5 μ g IgE SUS11 or PS, 0.5 μ g IgE ND, respectively, were applied to the gel. The gel was run at room temperature at 100 V. The gel was stained with 0.1% (w/v) Coomassie blue R250 (Fluka) in 10% (v/v) acetic acid and destained with 40% (v/v) methanol, 10% (v/v) acetic acid.

For Western blot proteins were transferred to nitrocellulose (pore size: 0.2 μ m; BA83 401396, Schleicher and Schuell, Riehen, Switzerland), washed with TBS,

pH 7.4 and blocked with 1.5 mg/ml casein in TBS for 1 h. Then nitrocellulose was incubated overnight with 5 ml (3 μ g/ml) POX-labeled anti-IgE antibody (Le27). After washing with TBS (3 times for 10 min) blots were developed with chloronaphthol (0.25 mg/ml) and 0.04% H_2O_2 (v/v).

4. Results

4.1. The TTd-specific response in the CD40 system

It is known that in the CD40 system large amounts of all 4 secreted isotypes of Ig can be induced [32]. Furthermore it was claimed that the CD40 system might represent an in vitro approximation for events taking place in germinal centers [14]. In view of this we first studied the TTd-specific response in the CD40 system as a model. Therefore cells were cultured in the CD40 system and subsequently immortalized by fusion with LA55 cells.

In Table 1 our results are compared with data previously published by ourselves or other groups. It shows that by using various experimental approaches TTd-specific clones can be easily generated. Anti-TTd specific clones were even found if spleen cells or PBL from donors were used which had not recently been boosted, though at a lower frequency than by cells from

TABLE 1

GENERATION OF TETANUS TOXOID (TTd)-SPECIFIC HYBRIDOMAS WITH DIFFERENT CULTURE AND IMMORTALIZATION SYSTEMS

Culture				Clonal analysis			Ref.
Immuniz. with TTd	Selection procedure	Culture system and immortalization (fusion partner)	Number of lymphocytes ($\times 10^6$) (source)	TTd-specific IgG clones (frequency $\cdot 10^{-7}$)	Non-specific IgE clones (frequency $\cdot 10^{-7}$)	TTd-specific IgE clones	
No	None	PWM(4d) (LCL-HF2)	50 (spleen)	4 (0.8)	ND ^a	ND	36
No	None	EBV transformation (no fusion)	46 (PBL)	8 (1.7)	ND	ND	Lang ^b
Yes	None	Direct fusion (Ag8.653)	6 (PBL)	5 (8.3)	ND	ND	37
Yes	None	Direct fusion (LCL-LTR228)	6 (PBL)	5 (8.3)	ND	ND	38
Yes	TTd-specific	EBV transformation (no fusion)	20 (PBL)	52 (26)	ND	ND	30
No	Antigen on MACS ^c	CD40 system. (LA55)	10 (PBL)	0	78 (780)	0	
Yes	CD19 on MACS ^d	CD40 system. (LA55)	5.5 (PBL)	0	12 (22)	0	

^a ND, not done.

^b Unpublished observation.

^c PBL were enriched for TTd specificity with biotin-labeled tetanus-toxin and streptavidin-beads in a MACS system.

^d PBL of a healthy volunteer were isolated 7 days after immunization with TTd. After 7 days of culture with IL-4 (50 U/ml) and anti-CD40 antibody (100 ng/ml) B cells were purified by positive selection using CD19 beads in a MACS system.

freshly boosted individuals. In contrast, if cells from an immunized donor were specifically selected by a TTd-specific panning procedure large numbers of specific clones were found after immortalization.

In our approach we also used a technique for enrichment of TTd-specific B cells (biotin-labeled TTd and streptavidin-beads in the MACS system). In contrast to the previous findings, no immortalized cells secreting TTd-specific Ig were obtained after culture in the CD40 system (Table 1). Screening after fusion showed that a large number of clones secreting IgE had been generated. But none of them was specific for TTd.

In order to confirm these findings a volunteer was immunized with TTd and blood was drawn 7 days later. To assure the presence of antigen-specific cells we cultured PBL with or without IL-4 and anti-CD40 antibody for different time periods and measured TTd-specific Ig levels in their supernatants. Fig. 1 shows that an ongoing specific antibody production, unaffected by the presence of IL-4 and anti-CD40 antibody, was

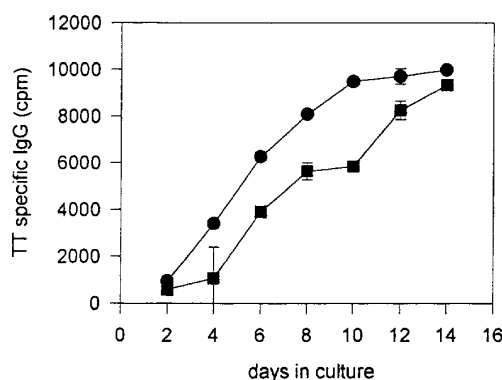


Fig. 1. Production of TTd-specific IgG by cells from a freshly boosted individual. PBL (1.5×10^6 /ml) were cultured in 1 ml cultures in IMDM without (○) or with (□) IL-4 (50 U/ml) and anti-CD40 antibody (100 ng/ml) for different time periods as indicated. TTd-specific IgG was detected using polystyrene plates coated with TT (10 μ g/ml) and an ^{125} I-labeled monoclonal anti-IgG antibody (BS17).

TABLE 2

TOTAL AND ALLERGEN-SPECIFIC IgE INDUCED BY ALLERGEN STIMULATION, IL-4 AND anti-CD40 ANTIBODY

Stimulation with		allergen ^a (ng/ml)	Total IgE (ng/ml $\pm$ SD)	Specific IgE ^b (ng/mg $\pm$ SD)
Donor 1	–	–	2 $\pm$ 1	0
	IL-4	–	172 $\pm$ 52	0
	anti-CD40	–	2 $\pm$ 1	0
	IL-4 + anti-CD40	–	439 $\pm$ 247	0.30 $\pm$ 0.71
	IL-4 + anti-CD40	1000	753 $\pm$ 141	0
	IL-4 + anti-CD40	500	835 $\pm$ 83	0
	IL-4 + anti-CD40	250	812 $\pm$ 50	0
Donor 2	IL-4 + anti-CD40	125	646 $\pm$ 176	0.09 $\pm$ 0.42
	–	–	0	0
	IL-4	–	9 $\pm$ 4	0
	anti-CD40	–	0	0
	IL-4 + anti-CD40	–	338 $\pm$ 42	0
	IL-4 + anti-CD40	1000	550 $\pm$ 32	0
	IL-4 + anti-CD40	500	501 $\pm$ 88	0
Donor 3	IL-4 + anti-CD40	250	338 $\pm$ 84	0.60 $\pm$ 0.39
	IL-4 + anti-CD40	125	365 $\pm$ 113	0
	–	–	2 $\pm$ 1	0
	IL-4	–	17 $\pm$ 4	0
	anti-CD40	–	4 $\pm$ 1	0
	IL-4 + anti-CD40	–	376 $\pm$ 88	0.20 $\pm$ 0.16
	IL-4 + anti-CD40	1000	144 $\pm$ 18	0.62 $\pm$ 0.13
	IL-4 + anti-CD40	500	161 $\pm$ 11	0
	IL-4 + anti-CD40	250	169 $\pm$ 30	0
	IL-4 + anti-CD40	125	305 $\pm$ 53	0

^a Cells were stimulated with a mix of extracts containing the major antigens of birch, 6 grasses and *D. pteronissinus*.

^b Specific IgE was detected in a RAST using the same allergens as for stimulation (see ^a). Bound IgE was estimated using the same standard curve as for total IgE.

clearly demonstrable. In parallel, after 1 week of culture in the presence of IL-4 and anti-CD40 antibody B cells were purified and transferred to the CD40 system and finally fused. As shown in Table 1, screening revealed similar results as in our first approach. No TTD-specific but again large numbers of IgE-secreting clones were generated.

4.2. Total and allergen-specific IgE response in the presence of allergen, IL-4 and anti-CD40 antibody

These data suggested that by stimulation with IL-4 and anti-CD40 in the CD40 system, despite the high levels of synthesized IgE, no preferential switch to IgE production by antigen-specific B cells was induced. We speculated that this apparent lack of isotype switch in TTD-specific cells might be due to the nature of the antigen. Therefore we chose allergens to eventually induce a specific IgE response. For this purpose cells isolated from 3 individuals suffering from allergies and with detectable specific serum IgE levels (RAST-class 3–4 for at least one of the allergens used, i.e., birch, mix of 6 grasses and *D. pteronissinus*) were stimulated with IL-4, anti-CD40 antibody and purified allergen-extracts. As expected high amounts of total IgE were synthesized by cells of all 3 donors as measured in the culture supernatants after 14 days (Table 2). Additionally very low amounts of specific IgE were measured but independent of the presence or concentration of the allergen. The large standard deviations of our results reflect the fact that only single cultures within the triplicates contained detectable levels of specific IgE.

As it has been claimed that antigen-specific cell activation and expansion were facilitated after depletion of monocytes and NK cells by treatment of cells with L-leucyl-methyl ester [29] we tested whether this method might be useful for our purpose to induce specific IgE. Again only single wells contained specific IgE while all wells showed elevated levels of total IgE in the presence of IL-4 and anti-CD40 antibody (data not shown). Taken together these data demonstrate that allergen-specific IgE producing B cells were not specifically expanded by stimulation with IL-4, anti-CD40 antibody and allergen. However, our data indicate that allergen-specific cells were present but only at very low numbers.

4.3. Generation of human IgE antibody-producing hybridomas after B-cell culture in the CD40 system

Thus we intended next to use the CD40 culture system, in which small numbers of cells can be clonally expanded, in order to grow out the few allergen-specific cells. Either total PBL or purified B cells from PBL

were cultured in the CD40 system for at least 14 days. After 2 or 3 weeks a clear increase of the total cell number was observed as documented by the amplification factor in Table 3. In addition substantial amounts of IgE were measured in culture supernatants at the end of the cell culture before fusion. IgE levels of positive cultures ranged from 10 to 300 ng/ml.

Cells were then fused with LA55 cells. As shown in Table 3 immortalization was efficient. In two fusions, growing hybridomas were found in all seeded wells. The frequency of hybridomas producing IgE was again high. Between 21% and 74% of the growth positive wells were found to contain more than 10 ng/ml IgE. In parallel these primary cultures were also screened for the content of allergen-specific IgE in a RAST. However, no specific IgE was detected in any culture. At this stage, cultures with high total IgE production (> 200 ng/ml) were selected and recloned at least twice by limiting dilution.

After recloning some selected clones of donor B, who was known to be allergic against grasses and birch, were nevertheless retested for specificity against birch- and a mixture of grass-allergens as well as other ubiquitous allergens yielding again negative results (data not shown).

4.4. Characterization of the human IgE-producing hybridoma clone SUS11

Among the established clones obtained we chose one for further characterization. Fig. 2 shows the results of a comparison of this clone (SUS11) with the human IgE-producing myeloma cell line U266. For U266 a mean cell-doubling time of 42.5 h was measured. Peak production of IgE was reached after 11 days of culture. The final IgE concentration was 8.7 µg/ml yielding an IgE production rate of 1.7 µg/10⁶ cells/24 h. In con-

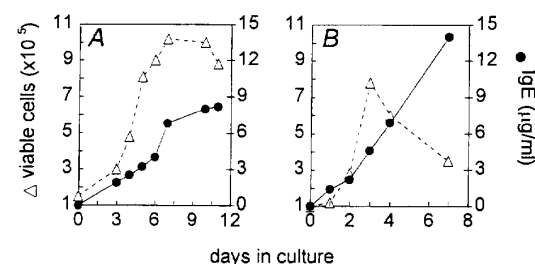


Fig. 2. Growth and IgE production of 2 human IgE-producing B-cell lines. Cells were cultured in 10 ml culture flasks at an initial density of 10⁵ cells/ml. A: U266 myeloma, cultured in RPMI-1640 supplemented with 10% FCS. B: SUS11 hybridoma, cultured in IMDM supplemented with 10% FCS. IgE was measured by RIA; cell viability was estimated by trypan-blue exclusion.

TABLE 3
GENERATION OF HUMAN IgE ANTIBODY-SECRETING HYBRIDOMAS

Exp	Culture Cellular source	Selection procedure	Cell number after selection (% of start)	CD40 system ^a		Immortalization			Specificity ^d
				Days in culture	Amplification factor	Cell number used for fusion ^b	Growth positive wells	IgE positive wells ^c	
A	PBL	none	8×10^5 (100%)	21	7.5	3×10^6	96/96	71/96	0/96
B	PBL	CD19 ^f	2.1×10^6 (7.7%)	21	2	2.1×10^6	96/96	53/96	0/96
C	PBL	CD19 ^f	6.3×10^5 (1.9%)	14	8.7	5.5×10^6	58/288	12/58	ND ^e

^a Cells were cultured in the presence of IL-4 (50 U/ml) and anti-CD40 antibody (500 ng/ml) on a monolayer of CD32 expressing Ltk-fibroblasts (2.5×10^3 /well).

^b Cells were fused at a 1:1 ratio with LA55 cells.

^c IgE was measured using a sandwich RIA. Wells were considered positive if supernatant contained more than 10 ng/ml IgE.

^d Specific IgE against the major allergens of birch, 6 grasses and *D. pteronissinus* was measured using a RAST as described in Materials and Methods.

^e Not done.

^f B cells were purified using the MACS system by positive selection with anti-CD19 antibody coupled to magnetic beads.

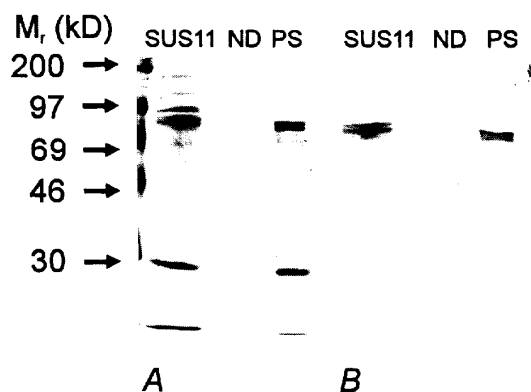


Fig. 3. SDS-PAGE and Western blot analysis of different purified IgE proteins. A: affinity-purified IgE from supernatants of the human B-cell lines SUS11 and U266 and IgE PS were analyzed by SDS-PAGE and Western blot run under reducing conditions. B: the proteins of a gel run as described in (A) were transferred to nitrocellulose. POX-labeled anti-IgE antibody (Le27) was used for detection of IgE on Western blot.

trast SUS11 cells had a mean doubling time of 19 h and IgE peak production was observed already after 7 days of culture. A final concentration of 13.2 $\mu\text{g}/\text{ml}$ was achieved resulting in an IgE production rate of 4.5 $\mu\text{g}/10^6\text{cells}/24\text{ h}$. The SUS11 clone is now in culture for more than 8 months with stable IgE production.

Purified IgE-SUS11 was compared to myeloma IgE-ND and IgE-PS on SDS-PAGE and Western blot analysis (Fig. 3). The same pattern for myeloma and hybridoma IgE was obtained. These results indicate that SUS11 produces complete human IgE. The function of IgE-SUS11 was tested by sensitizing Fc ϵ RI-transfected CHO-cells with different types of IgE. IgE-SUS11 and IgE-ND behaved similarly in this assay (data not shown). Taken together we show that it was possible to generate stable IgE-producing hybridomas thus providing a novel source of human IgE.

5. Discussion

Despite the many reports on total IgE synthesis in vitro there are only few on allergen-specific IgE synthesis. One report suggested that addition of relevant allergen even suppressed synthesis and release of allergen-specific IgE [16]. Another study on allergen specific IgE response assessed by ELISPOT-technique demonstrated that the frequency of allergen-specific B cells was only marginally elevated by addition of allergen, IL-4 and T cells [18]. Therefore we intended to use a B-cell culture system that allows significant expansion of B-cell clones in order to study in vitro synthesis of specific IgE.

By promoting survival of germinal center B cells (IgD $^-$ CD39 $^-$) CD40 seems to play a crucial role in the secondary response [33,34]. Activation of CD40 either by anti-CD40 antibody or activated T cells is mandatory for the induction of an antigen-specific response in vitro [35]. Furthermore it was reported that in the CD40 system both, naive (IgD $^+$) and mature (IgD $^-$) cells were expanded [14]. However, even though the combination of IL-4 and anti-CD40 antibody induces large amounts of IgE in vitro, it has not been studied whether this strong IgE response includes allergen-specific B cells.

Therefore we tried to induce specific IgE with IL-4, anti-CD40 antibody and allergen-extracts. Occasionally we measured small amounts of specific IgE but only in individual cell culture supernatants which also explains the large standard deviations in Table 2. We assumed that specific cells were present in the periphery even though at very low numbers. In the literature it was speculated that the monomeric soluble form of the allergen could induce an anergic state of allergen-specific B cells [16]; but the low amounts of specific IgE measured in our experiments and the fact that we measured specific IgE also in absence of allergen and independent of the allergen concentration does neither allow to confirm nor to exclude a hypothetical B-cells anergy.

Even though PBL are not the optimal source for memory B cells, it is known that for human hybridoma generation they represent a suitable source for obtaining specific clones if the donor was immunized or naturally exposed to the antigen. Specific clones can even be obtained if the last immunization took place months before [36,37]. Therefore we assumed that it should also be possible to derive allergen-specific B cells producing IgG and rarely IgE from PBL of allergic donors.

It is well established that normally TTD-specific immortalized cells can easily be obtained with various culture and immortalization procedures, especially in our population which is widely immunized against this antigen (Table 1). Of course the number of clones may also vary from donor to donor in relation to the number of specific cells in the starting material and the efficiency of immortalization. This fact does not change our finding that after B-cell culture in the CD40 system specific cells are lost or overgrown. In addition to published information referred to in Table 1, one of the authors (A. Lang) has experimental experience in obtaining TTD-specific hybridomas over many years, during which it did not occur that no specific clones were derived from freshly immunized donors. Our pilot-experiments (Fig. 1) indicated that the lack of TTD-specific clones was not due to the absence of specific cells in the starting material but rather to the fact that antigen-

specific cells were selectively overgrown in the CD40 system. These findings confirm a recent report by Galibert et al. [23] documenting that IgD⁻/IgM⁻ cells can be grown out only to a limited extent and are eventually lost in culture because they are overgrown by IgD⁺/IgM⁺ cells [23]. Probably the few allergen-specific B cells secreting IgE were present only during the early stages of the CD40 system culture and were subsequently overgrown by naive cells. However, our most interesting finding is the lack of TTd-specific B-cell growth in the CD40 system. Here the argument of low precursor frequency cannot be raised as in the case of allergen-specific B cells. Obviously there occurred also no sequential switch to IgE in the specific B-cell population else we would have detected TTd-specific IgE-producing cells. Thus, the importance of our negative results is to demonstrate that the CD40 system may not be suitable for the study of a secondary Ig response in vitro.

For immortalization of human B cells the cell line LA55 has been proven to be a potent fusion partner. Specific human hybridomas of different isotypes were generated with this fusion partner [27,30]. Indeed, by using LA55 and CD40 system derived B cells we were capable of generating with high efficiency stable IgE-secreting hybridomas. To our knowledge this is the first description of generating human IgE hybridomas. Several of these hybridomas serve now in our laboratory as a valuable source of human IgE since the cell lines stably produce significant IgE levels in bulk cultures. Thus, despite the fact that the CD40 system is not suitable for expanding antigen-specific B cells it represents a good tool for culture of cells which can be fused to yield non-specific IgE-producing hybridomas.

Nevertheless, for the understanding of the allergic response it is still mandatory to develop allergen-specific Ig-synthesis systems in vitro. As shown here IL-4 alone is obviously not potent enough to expand the specific B cells to a degree that significant immunoglobulin levels can be determined. Thus one may speculate that either cofactors other than CD40 and/or other cytokines must be added allowing a culture system for antigen-specific IgE synthesis. Provided that such expansion systems for specific B cells will be available in the future it will be easy to obtain monoclonal antigen-specific IgE by using our fusion protocol.

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