Brief Definitive Report

CD40 and IgE: Synergism between Anti-CD40 Monoclonal Antibody and Interleukin 4 in the Induction of IgE Synthesis by Highly Purified Human B Cells

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Summary

A novel pathway of IgE–B cell differentiation has been identified. Engagement of the B cell antigen CD40 by $F(ab')_2$ fragments of monoclonal antibody (mAb) 626.1 in the presence of recombinant interleukin 4 (rIL-4) induced intense IgE synthesis, but modest IgG synthesis, by highly purified human B cells. Surface IgE^- B cells isolated by cell sorting were induced to produce IgE by mAb 626.1 and IL-4. Thus, IgE synthesis is unlikely to result from expansion of a B cell population precommitted to IgE in vivo. A neutralizing anti-IL-6 antibody strongly, but not completely, inhibited the IgE response. This indicates that autocrine production of IL-6 plays an important amplification role in IgE synthesis triggered by anti-CD40 mAb and IL-4. Although the exact role played by CD40 in IgE responses in vivo remains to be established, this T cell–independent system represents a useful model to characterize the biochemical and molecular events leading to IgE synthesis in human B cells.

Recent intense investigations have revealed the complexity in the induction of IgE synthesis. In man, the important role played by IL-4 has been extensively documented in that this cytokine appears to generate an essential signal for this process (1). However, this signal is not sufficient for resting human B cells to initiate IgE synthesis (2, 3). In this regard, activation signals generated in either allogeneic or autologous MLR (2, 4) and in a T cell–independent EBV infection (5, 6) are synergistic with IL-4. Both MLR and EBV infection are complex processes and are not easily amenable to further analysis.

In an attempt to develop a T cell–independent system to investigate the signal requirements for the induction of IgG synthesis, we have screened a series of mAb to B cell surface antigens to determine if they were synergistic with IL-4 to induce IgE synthesis by human B cells. Anti-CD40 mAb 626.1 has been found to potently synergize with IL-4 to induce IgE synthesis. As in other IgE synthesis systems, endogenous IL-6 also plays an important role.

Materials and Methods

Reagents. $F(ab')_2$ fragments of mAb 626.1 (IgG1 anti-CD40) were obtained as previously described (7). The sources for mAb L243 (anti-HLA-DR), anti-Leu-16 (anti-CD20), OKB7 and HB5 (anti-CD21), anti-μ antibody, rIL-4, rIL-6, and a neutralizing goat IgG anti-human IL-6 antibody have been previously described (2, 6, 8).

B Cell Cultures. Highly purified human B cells from nonatopic subjects were prepared as previously described (2). The cultures (10^6 B cells/ml) were set up as described in Results. Supernatants were assessed by RIA for their IgE content, and by ELISA for IgM and IgG concentrations, as previously described (3). Control cultures for the evaluation of preformed Ig were set up in the presence of cycloheximide (100 μg/ml) (Sigma Chemical Co., St. Louis, MO).

Cell Sorting. Purified B cells were labeled with FITC-conjugated affinity-purified goat anti-human IgE antibody (Tago Inc., Burlingame, CA), or with PE-conjugated anti-CD20 mAb, and sorted on a FACStar Plus flow cytometer (Becton Dickinson & Co., Mountain View, CA). Setting of the markers and analysis of the sorted cells were performed as previously described (6).

Results

In an attempt to identify surface antigens that are important in IgE synthesis, antibodies to B cell–specific antigens were used in conjunction with IL-4 to induce B cells to synthesize IgE. Antibodies to surface IgM, CD20, CD21, and MHC class II molecules were found to be ineffective (data

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not shown). However, anti-CD40 mAb 626.1 was identified to be a potent agent in this system. As shown in Table 1, no IgE production was detected in cultures stimulated with either anti-CD40-F(ab')$_2$ or IL-4 alone. In contrast, substantial production of IgE was consistently observed when both rIL-4 and anti-CD40 mAb were added. Similar results were obtained in four experiments with different B cell donors. Optimal IgE production was obtained after 10 d of culture, using IL-4 at 100 U/ml and mAb 626.1 at 5 μg/ml. Although anti-CD40 mAbs have been shown to rescue B cells from death in culture (9), cell viability was not significantly increased in cultures stimulated with mAb 626.1 and IL-4, as compared with cultures receiving IL-4 alone (data not shown).

The B cell preparations we used had been rigorously depleted of T cells, as indicated by the absence of proliferation to PHA. Furthermore, IL-4 and anti-CD40 mAb induced IgE synthesis in B cells isolated by positive sorting for CD20 expression (Table 1, Exp. 2). These data indicate that the production of IgE induced by IL-4 and CD40 engagement by mAb is T cell independent.

We next investigated the effect of mAb 626.1 and IL-4 on the synthesis of isotypes other than IgE. Table 2 shows that stimulation of purified B cells with anti-CD40 mAb, in the absence of IL-4, resulted in modest IgG synthesis, but no IgE or IgM synthesis. When both anti-CD40 mAb and IL-4 were added, IgG production increased only slightly further, while large amounts of IgE were synthesized. Thus, the synergism between IL-4 and CD40 engagement results primarily in the induction of IgE synthesis.

To rule out the possibility that the induction of IgE synthesis by IL-4 and anti-CD40 mAb may primarily result from the expansion of a small sIgE$^+$ B cell population that has undergone Cε switching in vivo (10), purified peripheral blood B lymphocytes were sorted into sIgE$^+$ and sIgE$^-$ cells using an FITC-conjugated affinity-purified goat anti-IgE antibody. sIgE$^-$ B cells were subsequently stimulated with mAb 626.1 and rIL-4. The fluorescence profile obtained in a representative sorting experiment is shown in Fig. 1. To avoid ambiguity, gates for sorting were set so that even the most dimly stained sIgE$^+$ cells were excluded. Upon reanalysis, no sIgE$^+$ cells were detectable in the sIgE$^-$ cell population. Fig. 1, *inset*, shows that rIL-4 and anti-CD40-F(ab')$_2$ induced sIgE$^-$ cells to produce large amounts of IgE. These results indicate that sIgE$^-$ B cell precursors are targets for IgE induction by IL-4 and anti-CD40 mAb.

We have previously shown that endogenous IL-6 plays an obligatory role in the IL-4-dependent induction of IgE synthesis by human PBMC. A neutralizing anti-IL-6 antibody completely inhibited IgE synthesis induced by IL-4 (3). Because B cells produce IL-6 after stimulation with either IL-4 (11) or anti-CD40 mAb (9), the role of endogenous IL-6 was assessed in our system. Table 3 shows that a neutralizing goat anti-IL-6 antibody, but not a control goat serum, strongly, although not completely, inhibited IgE synthesis by human B cells stimulated with mAb 626.1-F(ab')$_2$ plus IL-4. Inhibition by anti-IL-6 antibody was specific, because it was reversed by addition of rIL-6 (300 U/ml). These results show that autocrine production of IL-6 is an important step in the augmentation of IgE synthesis induced by IL-4 and anti-CD40 mAb.

Table 1. *Engagement of CD40 in the Presence of rIL-4 Induces T Cell–independent Synthesis of IgE by Purified Human B Cells*

Stimulant	Net IgE Synthesis	
	Exp. 1	Exp. 2*
	pg/ml	
Nil	<150	<150
rIL-4	<150	<150
anti-CD40 mAb-F(ab')$_2$	<150	<150
rIL-4 + anti-CD40 mAb-F(ab')$_2$	40,000	12,200

* Highly purified human B cells isolated by sorting for CD20 expression.

Table 2. *Induction of Ig Isotypes Synthesis by rIL-4 and Anti-CD40 mAb*

Stimulant	Net Ig Synthesis		
	IgM	IgG	IgE
		ng/ml	
Nil	54 ± 30*	55 ± 11	<0.15
rIL-4	14 ± 10	40 ± 11	<0.15
anti-CD40 mAb-F(ab')$_2$	76 ± 40	291 ± 146	<0.15
rIL-4 + anti-CD40 mAb-F(ab')$_2$	40 ± 10	441 ± 128	43 ± 19

* Mean ± SE of results obtained in four experiments.

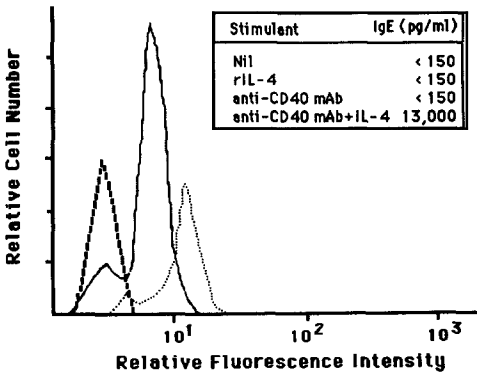

Figure 1. Flow cytometry profile obtained by staining highly purified B cells with FITC-conjugated affinity-purified goat anti-human IgE antibody (·····), or FITC-conjugated normal goat serum (——). Analysis for sIgE$^+$ cells in the sIgE$^-$ population isolated by sorting is also shown (– – –). (*Inset*) IgE production by sIgE$^-$ B cells obtained by cell sorting in a representative experiment.

Table 3. *Anti-IL-6 Antibody Inhibits the Synthesis of IgE Induced by anti-CD40 mAb and rIL-4*

Stimulant	Net IgE synthesis	
	Exp. 1	Exp. 2
	pg/ml	
Nil	<150	<150
rIL-4	<150	<150
anti-CD40 mAb-F(ab')$_2$	<150	<150
rIL-4 + anti-CD40 mAb-F(ab')$_2$	54,450	45,700
rIL-4 + anti-CD40 mAb-F(ab')$_2$ + anti-IL-6 antibody	3,600	4,250
rIL-4 + anti-CD40 mAb-F(ab')$_2$ + control serum	52,000	38,300

Discussion

This report describes a novel pathway of human B cell differentiation into IgE production, which is triggered by the engagement of the B cell antigen CD40 in the presence of IL-4. Induction of IgE synthesis in this system is T cell independent and primarily involves sIgE$^-$ B cells.

IL-4 has been shown to induce germ-line Cε transcripts in both human (6, 12) and murine (13) B cells. This is thought to reflect an increase in the accessibility of the ε switch region, which would become the target for switch recombination. The exact role of IL-4 in IgE isotype switching remains to be identified. Induction of IgE synthesis by anti-CD40 mAb plus IL-4 may provide a well-defined system to characterize the biochemical and molecular events that underlie isotype switching.

CD40 is a 50-kD surface glycoprotein expressed on all human B lymphocytes, but not on T cells and monocytes (14). Anti-CD40 mAbs (such as mAbs G28.5 and S2C6) deliver a strong progression signal to resting B cells in the presence of primary activators (14). More recently, our anti-CD40 mAb 626.1 has been shown to induce significant proliferation of resting B cells in the absence of other costimuli (7). Cloning of the CD40 gene has revealed that CD40 is closely related to the receptors for nerve growth factor (15) and TNF-α (16). These homologies and the growth factor–like activity of anti-CD40 mAb suggest that CD40 may be involved in the regulation of B cell activation and growth. Thus far, the ligand for CD40 has not been identified. The possibility that it may be involved in cell-cell interactions must be considered. With the identification of this ligand, the exact role of CD40 in B cell activation in general, and in IgE synthesis in particular, can be elucidated.

It is not clear at which step(s) the IL-4- and CD40-dependent signaling pathways actually synergize to induce IgE production. IL-4 has been shown to costimulate human B cells only when combined with competence signals that activate PKC and induce increase in intracellular calcium (17). Anti-CD40 mAb 626.1, however, does not induce calcium mobilization, even after crosslinking (7). Interestingly, IL-4 has been shown to upregulate CD40 expression on B cells (17). This upregulation may amplify the signals generated by the reaction between CD40 and the mAb 626.1, and may be important for IgE induction.

Endogenous IL-6 has been shown to be required for the T cell–dependent induction of IgE synthesis by human PBMC (3). Our data suggest an important amplification role of IL-6 in IgE synthesis induced by IL-4 and CD40 engagement. Interestingly, both IL-4 and anti-CD40 mAb induced IL-6 production in B cells (9, 11). Furthermore, IL-6 increases the phosphorylation of CD40 in human B cells, thus leading to a CD40-IL-6 loop (9). IL-6 is known to act at a late stage in B cell differentiation with no isotype preference. IL-6 induces the secretion of IgG1 by coordinated transcriptional activation, selective accumulation of mRNA for the secreted form of IgG, and possibly differential mRNA stabilization (18). IL-6 may amplify IgE secretion by similar mechanisms.

In mice, T cell–independent synthesis of IgE can be readily induced by stimulation with LPS and IL-4. In contrast, all polyclonal B cell activators tested (anti-IgM antibodies, *Staphylococcus aureus* Cowan I, phorbol esters, anti-CD20 mAb), with the exception of EBV, have failed to synergize with IL-4 in the induction of IgE synthesis by purified human B cells (1, 2, 5, 6). Although mAbs to the EBV receptor synergize with anti-IgM antibodies to induce B cell activation (19), they were unable to mimic EBV in synergizing with IL-4 for IgE induction (6). Thus, to our knowledge, the system described herein is the first one in which the B cell activating signal required for human IgE synthesis is delivered by engagement of a discrete B cell surface antigen. This further underscores the significance of the role of CD40 in IgE synthesis.

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