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CD40 plays an essential role in the activation of human B cells by murine EL4B5 cells

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SUMMARY

A mutant subclone of the murine thymoma EL-4, known as EL4B5, can strongly activate human B cells to proliferate and differentiate in a cell–cell contact-dependent manner. We have investigated whether interaction via CD40 plays a role in this helper activity. For this purpose, three newly generated anti-CD40 monoclonal antibodies (mAb) were used. In contrast with other anti-CD40 mAb described in the literature, these mAb did not co-stimulate proliferation of human B cells. On the other hand, these novel mAb could inhibit the co-stimulatory effect of the previously described anti-CD40 mAb S2C6 on anti-IgM-induced human B-cell proliferation. It was found that addition of these non-stimulatory anti-CD40 mAb could completely inhibit EL4B5-induced human B-cell proliferation. Maximal inhibition occurred already at a mAb concentration of 10 ng/ml. Similarly, a fusion protein, consisting of the extracellular portion of CD40 and human IgM constant domains CH_2, CH_3 and CH_4, could completely inhibit EL4B5-induced human B-cell proliferation. Induction of human B-cell proliferation by EL4B5 cells was also inhibited by anti-CD40 mAb S2C6 and G28.5, but less effectively. In contrast, mAb against B-cell surface antigens CD20 or B7 had no inhibitory effects. It is concluded that interaction via CD40 is essential for the induction of human B-cell proliferation by EL4B5 cells.

INTRODUCTION

A few years ago, Zubler *et al.*[1] observed that a mutant subclone of the mouse thymoma EL-4 line, known as EL4B5, could strongly stimulate B cells from both murine and human origin to proliferate and differentiate *in vitro*. This activation was found to be antigen independent and major histocompatibility complex (MHC) non-restricted. For optimal stimulation of human B cells, the presence of supernatant from activated human T cells was needed; but a B-cell response also occurred when EL4B5 cells were preactivated with phorbol 12-myristate 13-acetate (PMA) or interleukin-1 (IL-1).[2,3] B-cell activation in this culture system is highly efficient: limiting dilution experiments have shown that the majority of human B cells can be activated to proliferate and differentiate into antibody-secreting cells.[4] The mean clone size of progenators derived from one single B cell was 380 and was reached after only 11 days of culture.[4]

Because of the high frequency of outgrowth of human B cells in this culture system, we have applied it in combination with electrofusion to generate human monoclonal antibodies (mAb).[5] The mechanism by which these mutant EL-4 cells activate both murine and human B cells has until now not been elucidated. It is, however, clear that cell–cell contact is required for EL4B5-induced B-cell activation. First, B cells do not proliferate in the presence of supernatant from PMA-stimulated EL4B5 cells.[1] Second, B cells do not proliferate when they are separated from PMA-treated EL4B5 cells by a semipermeable filter membrane.[3] However, the molecular basis of the EL4B5 helper activity is unknown. Antibodies against mouse lymphocyte function-associated antigen-1 (LFA-1), human LFA-1 or human LFA-3 and antibodies against mouse or human MHC class II molecules did not inhibit EL4B5-induced proliferation of human or murine B cells.[2,3]

Anti-CD40 mAb have been shown to be able to mimic effects of T-helper cells in B-cell activation. When presented on adherent cells expressing FcγRII, anti-CD40 mAb can induce B-cell proliferation even in the absence of other stimuli.[6] More-

Abbreviations: FITC, fluorescein isothiocyanate; FCS, foetal calf serum; hCD40.Hμ, fusion protein of soluble human CD40 and human IgM constant domains CH_2, CH_3 and CH_4; IMDM, Iscove's modified Dulbecco's medium; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate; PHA, phytohaemagglutinin; SRBC, sheep red blood cells.

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over, anti-CD40 mAb can replace the T-helper signal for secretion of IgM, IgG and IgE in the presence of IL-4.[7] Therefore, we have investigated whether interaction via CD40 plays a role in EL4B5-induced proliferation of human B cells. For this purpose, we used novel anti-CD40 mAb which were recently generated by immunizing mice with insect cells expressing recombinant CD40.[8] We show that these mAb, in contrast with the already existing anti-CD40 mAb S2C6[9] and G28.5,[10] do not stimulate proliferation of human B cells. Therefore we judged that these mAb were very suitable for investigating the effects of blocking CD40 on the stimulation of human B-cell proliferation by EL4B5 cells. Furthermore, to block a putative CD40 ligand on EL4B5 cells, a fusion protein consisting of the extracellular domain of human CD40 and human IgM constant domains CH_2, CH_3 and CH_4 (hCD40.Hμ)[11] was used.

MATERIALS AND METHODS

Cell lines

The mutant mouse thymoma EL-4 subclone EL4B5[4] was a gift from Dr R.H. Zubler (Hôpital Cantonal Universitaire, Geneva, Switzerland). Mouse 3T6 transfectant cells expressing hybrid molecules of the LR (low responder) and HR (high responder) allelic forms of human FcγRIIa were a gift from Dr P.A.M. Warmerdam (Department of Experimental Immunology, University Hospital Utrecht, Utrecht, The Netherlands).[12] Both cell lines were cultured in Iscove's modified Dulbecco's medium (IMDM), supplemented with gentamycin (80 μg/ml) and 10% heat-inactivated foetal calf serum (FCS; Hyclone, Logan, UT). To avoid possible loss of B-cell activating capacity,[1] every 4–8 weeks a new batch of EL4B5 cells was thawed. The cell lines were periodically tested for mycoplasma contamination by the use of a ^{3}H-labelled DNA probe for mycoplasma ribosomal RNA (Gen Probe, San Diego, CA) and were free of mycoplasma during the course of the experiments.

Antibodies and hCD40.Hμ fusion protein

Anti-CD40 mAb 5D12, 3C6 and 3A8 were generated by immunizing mice with insect cells expressing recombinant human CD40.[8] Anti-B7 mAb B7-24 was generated in a similar way by immunizing with insect cells expressing recombinant human B7.[8] Anti-CD40 mAb S2C6[9] was a gift from Dr S. Paulie (University of Stockholm, Sweden) and anti-CD40 mAb G28.5[10] was donated by Dr J. A. Ledbetter (Oncogen Corporation, Seattle, WA). As control antibodies the following were used: anti-β-glucocerobrosidase mAb 8E4 (IgG1),[13] myeloma immunoglobulins MOPC-21 (IgG1) and MOPC-141 (IgG2b) (Sigma, St Louis, MO), and purified human IgM (Sigma). All mAb were used as purified antibody preparations. The hCD40.Hμ fusion protein[11] was a gift from Dr P. Lane (Basel Institute for Immunology, Basel, Switzerland) and was used as a concentrated supernatant of transfected J558L cells. An estimate of the concentration of hCD40.Hμ in the supernatant was made by comparing the absorbance obtained in an ELISA specific for human IgM[5] with that of a known human IgM standard.

Isolation of human B lymphocytes

B lymphocytes were isolated from tonsils obtained from children undergoing tonsillectomy, essentially as described previously.[14] Briefly, the tissue was dispersed with scalpel blades, phagocytic and natural killer (NK) cells were depleted by treatment with 5 mM L-leucine methyl ester and T cells were removed by one cycle of rosetting with sheep erythrocytes (SRBC) treated with 2-aminoethyl isothiouronium bromide. The purity of the resulting B-lymphocyte preparations was checked by indirect immunofluorescent labelling with anti-CD20 mAb B1 (Coulter Clone, Hialeah, FL) or anti-CD3 mAb OKT3 (Ortho, Raritan, NY) and a fluorescein isothiocyanate (FITC)-conjugated F(ab')$_2$ fragment of rabbit anti-mouse Ig (Zymed, San Francisco, CA), and FACS analysis. The B-cell preparations contained (mean $\pm$ SD of six isolations): 95 $\pm$ 4% CD20$^+$ cells and 2 $\pm$ 1% CD3$^+$ cells.

Co-stimulation of B-cell proliferation with anti-CD40 mAb

B cells (4×10^4/well) were cultured in 200 μl IMDM supplemented with 10% FCS in flat-bottom 96-well microtitre plates. B cells were stimulated by the addition of immobilized anti-IgM antibodies (Immunobeads; 5 μg/ml; BioRad, Richmond, CA). Where indicated 100 U/ml recombinant IL-2 (a gift from Cetus Corporation, Emeryville, CA) was added. Varying concentrations of mAb were added at the onset of the microcultures and proliferation was assessed at day 3 by measurement of the incorporation of 0·3 μCi [^{3}H]thymidine after 18 hr pulsing.

Stimulation of B-cell proliferation by anti-CD40 mAb presented on FcγRII-expressing adherent cells

To test the ability of anti-CD40 mAb to stimulate B-cell proliferation in a culture system analogous to that described by Banchereau *et al.*,[6] mouse 3T6 transfectant cells expressing hybrid molecules of the LR and HR allelic forms of human FcγRII[12] were used. B cells (2×10^4/well) were cultured in flat-bottom microwells in the presence of 1×10^4 transfectant cells (irradiated with 5000 rads) in 200 μl IMDM supplemented with 10% FCS and 100 U/ml recombinant IL-4 (a gift from DNAX, Palo Alto, CA). Before addition of the B cells, the 3T6 cells were allowed to adhere to the culture plastic for at least 5 hr. Anti-CD40 mAb were added at concentrations varying from 15 ng/ml to 2000 ng/ml and proliferation of B cells was assessed by measurement of thymidine incorporation at day 7, upon 18 hr pulsing with 0·3 μCi [^{3}H]thymidine.

Activation of B cells with EL4B5 cells

B cells (1000/well) were cultured together with irradiated (5000 rads) EL4B5 cells (5×10^4/well) in flat-bottom microtitre plates in 200 μl IMDM supplemented with 10% heat-inactivated FCS, 5 ng/ml PMA (Sigma) and 5% human T-cell supernatant, unless otherwise indicated. Monoclonal antibodies were added at varying concentrations at the onset of the cultures and thymidine incorporation was assessed at day 6 after 18 hr pulsing with 0·3 μCi [^{3}H]thymidine.

For the preparation of T-cell supernatant, T cells were isolated from the SRBC rosette-forming cell fraction from tonsils by lysis of SRBC and were cultured at a density of 10^6/ml for 36 hr in the presence of 1 μg/ml PHA and 10 ng/ml PMA.[4] T-cell supernatant was obtained by centrifugation of the cells and stored at $-20°$. The effectiveness of T-cell supernatants in enhancing proliferation of human B cells in EL4B5 B-cell cultures was tested and the most effective supernatants were pooled and used in the experiments.

Flow cytofluorometric analysis of hCD40.Hμ binding to EL4B5 cells

EL4B5 cells were harvested before and at different time-points during culture in medium containing PMA (5 ng/ml) and human T-cell supernatant (5%). Cells were incubated for 30 min with 10 μl concentrated supernatant of transfected cells containing 0·35 μg hCD40.Hμ diluted in 100 μl Hanks' balanced salt solution supplemented with 0·05% NaN_3 (4°). This was followed by incubation with FITC-conjugated $F(ab')_2$ fragments of rabbit anti-human IgM (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands). As a control, cells were incubated with the FITC conjugate only. For analysis a FACScan 4 cytofluorometer (Becton Dickinson, Mountain View, CA) was used. Non-vital cells were excluded from analysis by the use of propidium iodide.

RESULTS

Functional properties of newly generated mAb against recombinant CD40 expressed on insect cells

Recently, we have expressed human CD40 in insect cells using the baculovirus expression system. By immunizing mice with these insect cells, four hybridomas producing mAb against human CD40 were generated.[8] The specificity of the mAb for CD40 has been established by blocking studies with soluble CD40. Furthermore, these mAb bound to a similar proportion of tonsil B cells as anti-CD40 mAb G28.5.[8] Three of these mAb (5D12, 3A8 and 3C6), which were of the IgG2b subclass, have now been tested for their ability to deliver activation signals to human B cells. First, it was found that none of these mAb was able to co-stimulate significantly human B-cell proliferation in the presence of immobilized anti-IgM or in the presence of immobilized anti-IgM and IL-2 (Fig. 1). In contrast, anti-CD40 mAb S2C6[9] co-stimulated human B-cell proliferation in a concentration-dependent fashion. Second, we investigated whether these mAb were able to induce proliferation of human B cells in a culture system similar to that described by Banchereau *et al.*,[6] i.e. by presenting the anti-CD40 mAb on adherent cells expressing FcγRII. As antibody presenting cells, mouse 3T6 transfectant cells expressing hybrid molecules of the LR and HR allelic forms of human FcγRII were used. These transfectants are capable of binding mouse IgG1, mouse IgG2a and mouse IgG2b.[12] It was observed that anti-CD40 mAb S2C6 together with IL-4 induced substantial proliferation of tonsillar human B cells in this system, as assessed by measurement of [^{3}H]thymidine incorporation. Anti-CD40 mAb 5D12, 3C6 or 3A8 however, did not induce proliferation of human B cells in this culture system (data not shown).

Since these mAb were not able to stimulate human B-cell proliferation, we next addressed the question whether these mAb could inhibit the co-stimulation of human B-cell proliferation by anti-CD40 mAb S2C6. It was found that each of the anti-CD40 mAb 5D12, 3A8 and 3C6 could inhibit the co-stimulation of anti-IgM-induced human B-cell proliferation by mAb S2C6 (Fig. 2). In contrast, no significant inhibition was seen with equivalent amounts of non-relevant mAb 8E4, directed to β-glucocerebrosidase.[13] Thus, it was concluded that these anti-CD40 mAb do not deliver stimulatory signals to the proliferation of human B cells, but, conversely, can inhibit stimulatory signals exerted by triggering CD40 with another

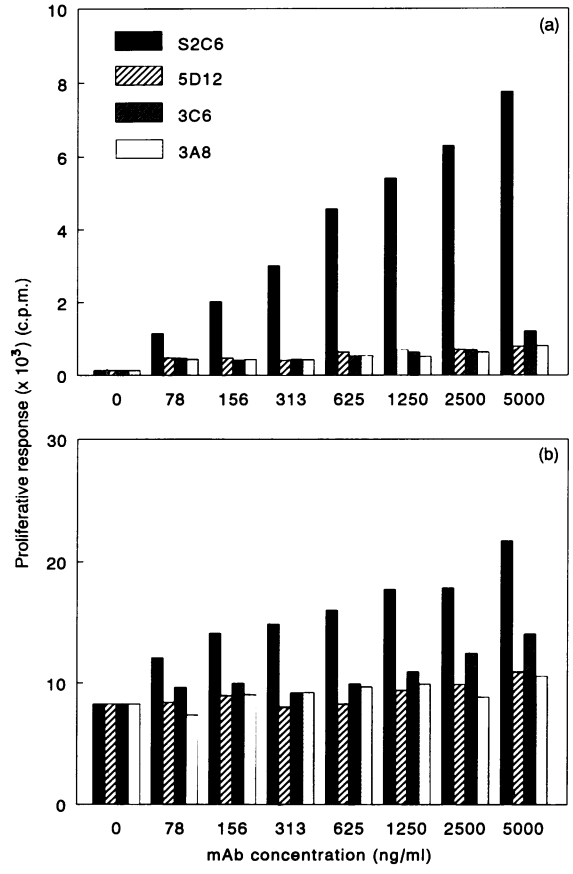

Figure 1. Ability of anti-CD40 mAb to co-stimulate anti-IgM-induced human B-cell proliferation. Human tonsillar B cells (4×10^4/well) were cultured in 200 μl in microwells in the presence of anti-IgM coupled to Sepharose beads (5 μg/ml) (a) or in the presence of anti-IgM plus rIL-2 (100 U/ml) (b). Varying concentrations of the anti-CD40 mAb S2C6, 5D12, 3C6 or 3A8 were added and [^{3}H]thymidine incorporation was estimated at day 3 after 18 hr pulsing. Data presented in (a) are means derived from experiments with B-cell preparations from three different donors with duplicate incubations. Data in (b) are means of duplicate incubations from one experiment out of two with comparable results.

mAb. Therefore, these mAb were considered to be excellent tools to investigate whether signalling via CD40 plays a role in the stimulation of human B-cell proliferation by EL4B5 cells.

Anti-CD40 mAb can completely inhibit EL4B5-induced B-cell proliferation

To investigate the effect of anti-CD40 mAb on EL4B5-induced human B-cell proliferation, human tonsillar B cells (1000/well) were cultured together with irradiated EL4B5 cells (50,000/well) in the presence of 5% supernatant of activated human T cells and 5 ng/ml PMA. Figure 3a shows that addition of anti-CD40 mAb 5D12, 3C6 or 3A8 resulted in a concentration-dependent inhibition of human B-cell proliferation. Very potent inhibition occurred, as each of the three anti-CD40 mAb 5D12, 3C6 and 3A8 inhibited human B-cell proliferation completely at concentrations as low as 10 ng/ml. Half-maximal inhibition was found at approximately 1 ng/ml. In contrast, isotype-matched IgG2b mouse myeloma protein MOPC-141 had no significant effect on

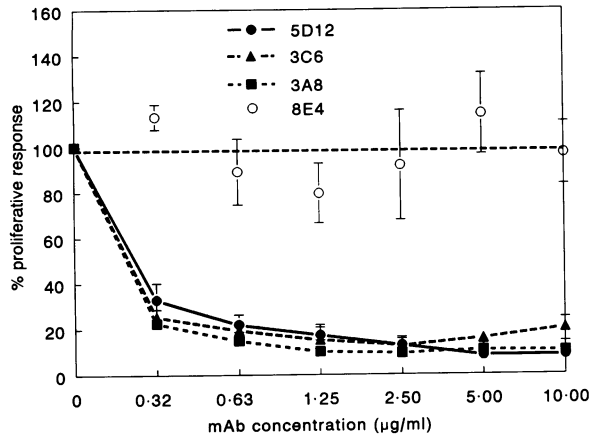

Figure 2. The novel anti-CD40 mAb can inhibit co-stimulation of immobilized anti-IgM-induced human B-cell proliferation by anti-CD40 mAb S2C6. Human tonsillar B cells (4×10^4/well) were cultured in 200 μl in microwells in the presence of anti-IgM coupled to Sepharose beads (5 μg/ml) and anti-CD40 mAb S2C6 (1·25 μg/ml). Varying concentrations of anti-CD40 mAb 5D12, 3C6 or 3A8 were added and [^{3}H]thymidine incorporation was assessed after 3 days. As a control anti-glucocerebrosidase mAb 8E4[13] was added in similar concentrations. Data are means ± SD derived from experiments with B cells from two different donors with duplicate incubations. [^{3}H]Thymidine incorporation in the absence of inhibitory antibody amounted to (means ± SD) 4964 ± 1311 and 2799 ± 530 c.p.m. in these two experiments respectively.

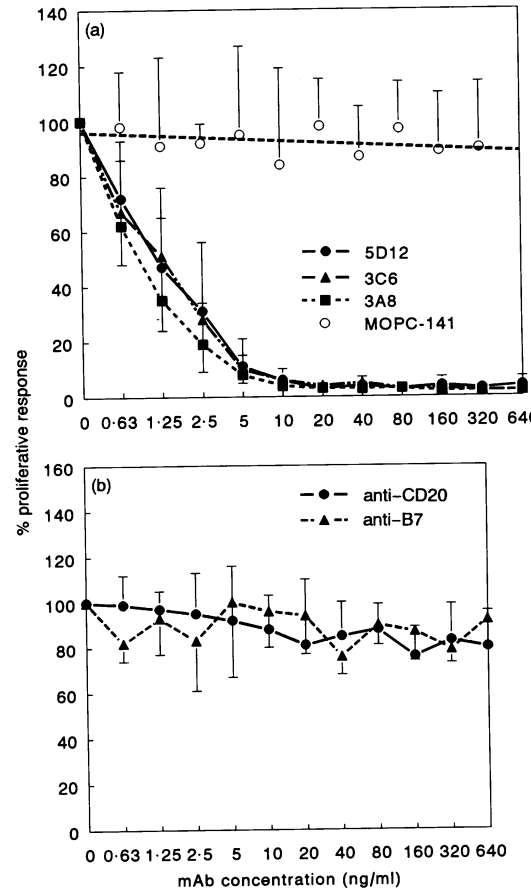

Figure 3. Novel anti-CD40 mAb 5D12, 3C6 and 3A8 but not anti-CD20 or anti-B7 mAb can inhibit EL4B5-inducd human B-cell proliferation. Human tonsillar B cells (1000 cells/well) were cultured together with irradiated EL4B5 cells (5×10^4/well) in the presence of PMA (5 ng/ml) and human T-cell supernatant (5%). In the experiments presented in (a) anti-CD40 mAb 5D12, 3C6 or 3A8 were added in varying concentrations and as a control mAb MOPC-141 (IgG2b) was used. In the experiments presented in (b) anti-CD20 mAb B1 or anti-B7 mAb B7-24 were added to the cultures. After 6 days of culture [^{3}H]thymidine incorporation was assessed. Data presented in (a) are means ± SD derived from experiments with B cells from four different donors with duplicate incubations. Values for [^{3}H]thymidine incorporation for incubations without mAb were (means ± SD) 10,460 ± 1843 c.p.m., 6982 ± 1729 c.p.m., 4362 ± 1020 c.p.m. and 15,430 ± 3190 c.p.m. in the four different experiments, respectively. Data in (b) are means ± SD from two experiments with duplicate incubations. Actual [^{3}H]thymidine incorporations in the absence of antibody in these experiments were 15,430 ± 3190 and 11,399 ± 1152 c.p.m., respectively. [^{3}H]Thymidine incorporation in B cells alone amounted to 40 ± 5 c.p.m. and in irradiated EL4B5 cells alone 31 ± 15 c.p.m.

[^{3}H]thymidine incorporation. The inhibitory effect of the anti-CD40 mAb was not caused by merely masking of the B-cell surface, since neither anti-CD20 mAb B1 nor anti-B7 mAb B7-24 (the latter mAb was generated by a procedure similar to that used for generating the novel anti-CD40 mAb),[8] had a significant effect on EL4B5-induced human B-cell proliferation (Fig. 3b). Furthermore, the observed inhibitory effect of the anti-CD40 mAb was not due to a change in the kinetics of the B-cell proliferation since similar inhibition was observed when [^{3}H]thymidine incorporation was assessed at day 4 of the culture instead of day 6.

EL4B5-induced human B-cell proliferation could also be inhibited by addition of the anti-CD40 mAb S2C6[9] and G28.5[10] (Fig. 4). However, none of these mAb was able to inhibit B-cell proliferation completely, which is probably due to a stimulatory effect of these mAb on human B cells. In particular S2C6 exerted much weaker inhibitory effects than the novel anti-rCD40 mAb used in the experiments reported in Fig. 3a.

A fusion protein of CD40 and human IgM can completely inhibit EL4B5-induced human B-cell proliferation

In order to investigate whether EL4B5 cells expressed a membrane structure which binds CD40, a fusion protein consisting of the extracellular domain of CD40 and human IgM constant domains CH_2, CH_3 and CH_4 (hCD40.Hμ)[11] was used for flow fluorocytometric analysis. Non-activated EL4B5 cells did not bind the fusion protein. However, upon culturing EL4B5 cells together with PMA (5 ng/ml) and 5% human T-cell supernatant, which are the conditions needed for activation of human B cells, a low binding of hCD40.Hμ was found (data not shown). This small shift in fluorescence was found consistently in three independent experiments. The minimal activation period needed for induction of the CD40 binding was 24 hr.

To determine whether binding of hCD40.Hμ to the EL4B5 cells would inhibit EL4B5-induced human B-cell proliferation as the anti-CD40 mAb did, the fusion protein was titrated into co-cultures of EL4B5 cells with human B cells. Figure 5 shows that the fusion protein did indeed inhibit [^{3}H]thymidine incorporation in a concentration-dependent manner, while human IgM had no effect. Like the novel anti-CD40 mAb, hCD40.Hμ could inhibit B-cell proliferation completely.

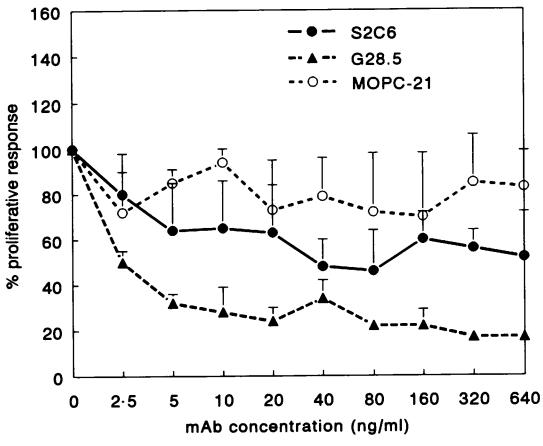

Figure 4. Effect of anti-CD40 mAb G28.5 and S2C6 on EL4B5-induced human B-cell proliferation. Human tonsillar B cells were cultured together with irradiated EL4B5 cells as described in Fig. 3. Varying concentrations of anti-CD40 mAb G28.5 and S2C6 were added. As an isotype-matched control mAb MOPC-21 (IgG1) was used. Data are means ± SD derived from three experiments with duplicate incubations. Actual [^{3}H]thymidine incorporations found in the absence of antibody were 6982 ± 1729, 4362 ± 1020 and 14,809 ± 2791 c.p.m. respectively.

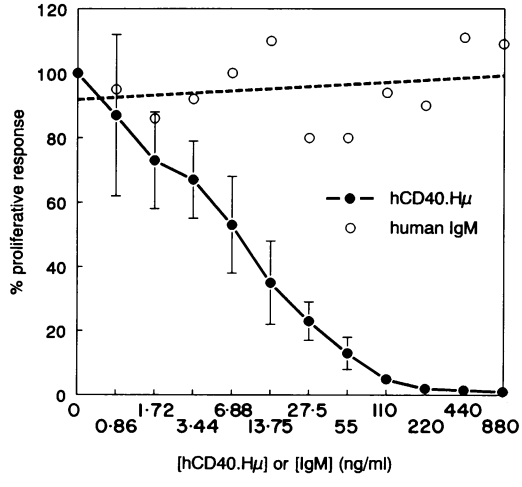

Figure 5. Effect of hCD40.Hμ on EL4B5-induced B-cell proliferation. Human tonsillar B cells were cultured together with irradiated EL4B5 in the conditions described in Fig. 3. A concentrated supernatant of transfectants secreting the hCD40.Hμ or purified human IgM was diluted sequentially in the culture medium and [^{3}H]thymidine incorporation was estimated after 6 days. The amount of hCD40.Hμ added is an estimation based on comparison with a known IgM standard in an ELISA specific for human IgM. Data for hCD40.Hμ represent means ± SD of four different culture experiments with duplicate incubations and data for human IgM means of one experiment with duplicate incubations. Actual [^{3}H]thymidine incorporation in the absence of hCD40.Hμ or IgM amounted to 6982 ± 1729, 4362 ± 1020, 14,809 ± 2791 and 11,399 ± 1152 c.p.m., respectively.

DISCUSSION

The data shown in this paper demonstrate that the helper activity of EL4B5 cells leading to the proliferation of human B cells can be completely inhibited by the addition of non-stimulatory anti-CD40 mAb or by the addition of a fusion protein consisting of human CD40 and the constant domains 2, 3 and 4 of human IgM. This implies that interaction via CD40 plays an essential role in the mechanism by which EL4B5 cells trigger human B-cell proliferation.

As has been explained above, the helper activity exerted by EL4B5 cells on B cells is strictly dependent on cell–cell contact. Therefore, it was plausible to propose that EL4B5 cells express a counter structure for CD40 on their surface. Indeed, a low binding of hCD40.Hμ was observed upon activation of the EL4B5 cells in medium containing PMA and human T-cell supernatant. Recently, Armitage *et al.*[15] demonstrated that the murine thymoma EL-4, from which the mutant EL4B5 has been derived, also bound human CD40, although with very low intensity. Upon several rounds of sorting, these authors isolated a subline with higher expression. From these cells they isolated and cloned a ligand for CD40 with a configuration which is typical for a transmembrane glycoprotein.[16] It is attractive to suppose that EL4B5 cells express the same molecule on their surface.

However, Armitage *et al.*[15] not only found expression of a CD40 binding structure on the surface of the sorted EL-4 cells, but also observed that these cells secreted biologically active soluble CD40 ligand. The inhibition of EL4B5-induced B-cell proliferation observed in the present study can, however, not be ascribed to inhibition of the binding of putative soluble CD40 ligand to the B cells. It has firmly been established that the helper activity of EL4B5 cells is dependent upon physical contact between the EL4B5 cells and the B cells.[1,3] Moreover, supernatant containing the secreted mouse CD40 ligand has been found not to stimulate proliferation of anti-IgM-activated human B cells.[15]

It cannot be excluded that supernatant derived from activated human T cells, which is added to the medium as a source of proliferation and differentiation factors, contains human soluble CD40 ligand. The observed inhibition of EL4B5-induced human B-cell stimulation is, however, not due to inhibition of the binding of a soluble human CD40 ligand to the B cells. In an additional experiment, it was found that the novel anti-CD40 mAb also could completely inhibit the (suboptimal) proliferation of human B cells induced by EL4B5 in the presence of PMA only, without the presence of human T cells supernatant (data not shown).

The magnitude of the inhibitory effect of anti-CD40 mAb on B-cell proliferation in the EL4B5 culture system may raise the question of whether these anti-CD40 mAb exert a generalized inhibitory effect on cells. This is not likely, as these mAb do not exert any inhibitory effect on B-cell proliferation induced by immobilized anti-IgM and IL-2 (Fig. 1b). Furthermore, the anti-CD40 mAb did not affect the vitality of (non-irradiated) EL4B5 cells, as assessed by eosin exclusion in an additional experiment.

In a recent paper, Noelle *et al.*[17] have demonstrated that activated murine T-helper clones transduce a signal for murine B-cell activation via CD40. Blocking of the interaction between the T cells and CD40 on the B cells by a fusion protein of CD40

and human immunoglobulin or by a mAb specific for the putative CD40 ligand on the murine T-helper cells inhibited the activation of the murine B cells by helper T cells. In the present study it is shown that activation of B cells of human origin by a murine T-helper cell line is strongly dependent on interaction via CD40. It is an intriguing question whether this is also an important mechanism by which human T cells regulate human B-cell activation. Recent data suggest that this may be the case.[11]

A second point which arises from this study is that the anti-CD40 mAb generated against recombinant CD40 expressed on insect cells have unique properties. In contrast to the previously described anti-CD40 mAb S2C6,[9] G28.5[10] and mAb89[18] these mAb do not co-stimulate human B-cell proliferation. As a consequence, only these novel mAb were able to inhibit EL4B5-induced B-cell proliferation completely. Inhibition was already exerted at picomolar concentrations. If T-cell-dependent human B-cell proliferation is regulated by interaction via CD40, these mAb may have important therapeutic applications for autoimmune diseases associated with exaggerated B-cell activation.

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