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Human CD40-ligand: molecular cloning, cellular distribution and regulation of expression by factors controlling IgE production

Jean-François Gauchat, Jean-Pierre Aubry, Gonzalo Mazzei, Paul Life, Therese Jomotte, Greg Elson and Jean-Yves Bonnefoy

Glaxo Institute for Molecular Biology, Plan les Ouates/Geneva, Switzerland

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Here we report the cloning of the cDNA for human CD40-Ligand (CD40-L) from a CD4-positive T cell clone. The deduced amino acid sequence predicts a type II membrane protein of 261 amino acids. Northern blot and FACS analysis of PBMNC revealed that the human CD40-L can be detected on T cells and is absent from B cells and monocytes. The human CD40-L is expressed on both CD4- and CD8-positive T cells, ($CD45R0^+$) and ($CD45RA^+$) subsets. We observed that IL-4, an inducer of IgE production, upregulated CD40-L mRNA level while IFNγ, an inhibitor of IgE synthesis, reduced the expression of CD40-L mRNA. These data suggest a the correlation between human CD40-L expression and IgE production.

CD40 ligand; Cloning; IFNγ; IgE regulation; IL-4; T cell

1. INTRODUCTION

The CD40 molecule is a glycoprotein expressed on B lymphocytes, epithelial cells and some carcinoma cells (for review see [1]). Cross-linking of CD40 by anti-CD40 monoclonal antibodies mediates B cell proliferation [2,3], adhesion [4], survival [5] and differentiation [6–9]. Recently, the murine cDNA for CD40-L has been cloned and was found to induce B cell proliferation as well as IgE production in the presence of IL-4 [10]. In this report we describe the cloning of the human CD40-L cDNA from a $CD4^+$ T cell clone library. The distribution and expression of the human CD40-L mRNA and protein were studied using a specific cRNA probe and a CD40-Fc fusion protein. The expression of CD40-L was examined under conditions which induce switching to the ε constant region and also IgE production.

2. MATERIALS AND METHODS

2.1. *Cells*

PBMNC were isolated by centrifugation over Ficoll-Hypaque and resuspended in Yssel's medium [11] supplemented with 10% foetal calf serum (FCS). The human CD4-positive T cell clones, JF7 and JF8 were raised by PHA/IL-2 stimulation of PBMNC according to the method of Yssel et al. [11]. The CD8-positive T cell clone, OZZ 50.1, is dog hair allergen-specific T cell clone (Life et al., manuscript in preparation). The T cell lines HuT-78, MOLT-4; the B cell lines, RPMI 8226 and IM-9; the promyelomonocytic cell line, HL-60; the histiocytic lymphoma cell line, U-937 and the monocytic cell line THP-1 were obtained from ATCC as was the BHK cell line. The Burkitt lymphoma cell line BL-2 was obtained from G. Lenoir (Lyon, France). RPMI 8866 and JY lymphoblastoid cell lines were obtained from K. Ishizaka (La Jolla, CA) and J. de Vries (Palo Alto, CA), respectively. The Jurkat cell line was obtained from A. Bernard (Nice, France). The murine cell line EL-4 was a gift of R. Zubler (Geneva, Switzerland). Cell lines were cultured in RPMI 1640 supplemented with 2 mM glutamine and 10% FCS. T cell clones were cultured in Yssel's medium supplemented with 10% FCS. Ionomycin and PMA were used at a final concentration of 1 μM and 10 ng/ml, respectively.

2.2. *Reagents*

Anti-CD40 mAb MCA 679 was purchased from Serotec Ltd (Oxford, UK). Control IgG_1 and IgM were obtained from Sigma (St. Louis, MO). IL-4 and IFNγ were purchased from Amersham International plc (Buckinghamshire, UK) and Genzyme (Boston, MA), respectively. The CD40-IgG_{2a} Fc fusion construct was a kind gift of I. Stamenkovic (Boston, MA) [12]. This was expressed in COS cells and purified as described [13]. Purified CD40-Fc was conjugated to FITC using the method of Titus et al. [14].

2.3. *Detection of the human CD40-Ligand using CD40-Fc*

Cells (4×10^5 in 40 μl) were incubated with 10 μg/ml purified FITC-conjugated recombinant CD40-Fc fusion protein or with FITC-IgG_{2a} (Becton Dickinson, Erembodegem, Belgium). For double staining, 4×10^5 cells were incubated with 10 μg/ml FITC-conjugated CD40-Fc fusion protein and 10 μg/ml phycoerythrin (PE-) labelled monoclonal antibodies directed against lymphocyte sub populations. Anti-CD3 (Leu 4a), -CD4 (Leu-3a+3b), -CD8 (Leu-2a), -CD14 (Leu-M3), -CD20 (Leu-16), -CD16 (Leu-11) and CD57 (Leu-7) were obtained from Becton Dickinson. Anti-CD45R0 (UCHL1) and anti-CD45 RA (2H4) were from Dako (Glostrup, Denmark) and Coulter (Hialeah, FL), respectively. After 30 min of incubation at 4°C, cells were washed in PBS containing 1% BSA and 2 mM NaN_3 and analysed on a

Correspondence address: J.-Y. Bonnefoy, Glaxo Institute for Molecular Biology, 14 ch. des Aulx, 1228 Plan les Ouates, Switzerland. Fax: (41) (22) 794 69 54.

Abbreviations: CD40-L, CD40 ligand; PBMNC, peripheral blood mononuclear cells; PMA, phorbol myristic acetate; PCR, polymerase chain reaction.

The sequence of CD40-L has been deposited in Genbank under accession number L 07414.

FACStar Plus flow cytometer (Becton Dickinson) equipped with an argon ion laser (Spectra Physics, Mountain View, CA) tuned at 488 nm. A compensation network was used to eliminate the FITC emission in the PE channel and the small contribution of PE in the FITC channel. Parameter settings were made using single labelled populations.

2.4. *Cell sorting of leukocyte sub populations by flow cytometry*

2×10^8 PBMNC were stained with optimal concentrations of FITC- or PE-labelled monoclonal antibodies directed against leukocyte sub populations. After cell sorting, an aliquot of each sorted population was re-analysed by flow cytometry and the purity of the cells was above 98%.

2.5. *Detection of the human CD40-Ligand mRNA with mouse CD40-Ligand cRNA probe*

Poly A^+ RNA was isolated from EL-4 cells using the guanidium thiocyanate-caesium trifluoroacetate (CsTFA) method [15] followed by oligo (dT)-cellulose chromatography [16]. cDNA was synthesised using a First-Strand cDNA synthesis kit (Pharmacia, Uppsala, Sweden). The *Hin*dII-*Bam*HI restriction fragment of the mouse CD40-L cDNA was amplified by polymerase chain reaction (PCR) [16] using the primers GGCGTTGACTCGAAGGCTCCCGATT and TGAGGATCCTCAAATTGCAGCAACAC and was cloned in pBC KS^+ (Stratagene, La Jolla, CA). The insert was checked by DNA sequencing [17]. The plasmid was linearized with *Bam*HI and used as a template to synthesise a cRNA probe [18]. For Northern blot assays, aliquots of RNA (2 μg) purified by the guanidium thiocyanate-CsTFA procedure were subjected to electrophoresis in 1% agarose, 6% formaldehyde gels [16], electrotransferred to nylon$^+$ membranes (Boehringer Mannheim, Mannheim, Germany), and fixed with UV irradiation [19]. Hybridisation with cRNA probes was performed at 57.5°C followed by 4 washes in 4 × SSC, 1% SDS at 65°C and a treatment with ribonuclease A as described previously [20]. Membranes were stained with methylene blue to localise and quantify rRNA before hybridisation [16].

2.6. *Cloning of the human CD40-Ligand cDNA*

Poly A^+ RNA was isolated from the JF7 $CD4^+$ human T cell clone using the guanidium thiocyanate-CsTFA procedure followed by two cycles of oligo (dT)-cellulose chromatography. cDNA was synthesised, linked to adapters and size selected using a TimeSaver cDNA synthesis kit (Pharmacia) according to the manufacturers instructions. The cDNA was cloned in the *Eco*RI restriction site of λgt10 and packaged using Gigapack II gold extract (Stratagene). One million of the 2.2 million plaque library (with more than 90% of phages containing inserts) were screened using the mouse CD40-L *Hin*dII-*Bam*HI restriction fragment labelled by random hexamer priming [16]. Hybridisation was carried out as described for Northern blot assays but with 15% formamide at 42°C and was followed by washes in 2 × SSC, 1% SDS at 55°C. Ten independent λgt10 clones were isolated by multiple screening cycles. Seven clones contained inserts which could be amplified by PCR using λgt10 specific primers. The inserts were recloned from purified λgt10 DNA into pBluescript II SK (Stratagene) and characterised by restriction mapping and sequencing of the insert ends [16]. The two *Eco*RI restriction sub fragments of clone 6 were recloned in M13 mp18 and mp19. The DNA sequence was determined on both strands by the dideoxynucleotide chain termination method [17].

2.7. *Transfection of the human CD40-L cDNA in BHK cells*

The 1.1 kb *Eco*RI restriction fragment of λgt10 clone 6 containing the open reading frame was recloned in pCDM8 [21]. Constructs with inserts in the two possible orientations were transfected into BHK cells by electroporation. Transfectants were analysed after 48 h of culture.

2.8. *Detection of the human CD40-Ligand mRNA with human CD40-ligand cRNA probe*

The 1.1 kb *Eco*RI restriction fragment of λgt10 clone 6 was recloned in pBluescript II SK. This plasmid, digested with the restriction enzyme *Hin*dIII was transcribed with T3 polymerase [18]. The human CD40-L cRNA probe was then used to detect the CD40-L mRNA by Northern blot assay using the hybridisation and washing conditions described in detail elsewhere [20]. Control Northern blot assays for actin mRNA were performed in parallel using a *Bam*HI restriction fragment of pHFγA1 [22] labelled by random hexamer priming as described [23].

2.9. *Induction of CD40-Ligand and IgE synthesis by IL-4 in PBMNC*

Cells were incubated at 10^6/ml for 14 days in Iscove's medium enriched with transferrin, bovine insulin, oleic acid, linoleic acid, palmitic acid, BSA (all from Sigma) and 10% FCS as described by Claassen et al. [24] in the presence of 200 U/ml recombinant IL-4. When added, IFNγ and anti-CD40 mAb were used at 500 U/ml and 1 μg/ml, respectively. Ig productions were measured by specific ELISA as previously described [25]. Control cultures for the evaluation of preformed Ig were set up in the presence of cycloheximide (100 μg/ml, Sigma).

3. RESULTS

3.1. *Cloning of human CD40-Ligand cDNA*

A cRNA probe complementary to a *Hin*dII-*Bam*HI restriction fragment of the murine CD40-L cDNA was used to detect the corresponding human mRNA using a Northern blot assay (Fig. 1A). This probe gave, as expected, a clear signal with RNA isolated from the murine EL-4 cell line. Reducing the hybridisation stringency led to the appearance of a band with an identical molecular weight in RNA isolated from the human $CD4^+$ clone JF7 which had been stimulated for 2 or 20 hours with ionomycin and PMA (Fig. 1A). No signal was detected in RNA isolated from unstimulated JF7 cells by cross hybridisation with the mouse cRNA probe. A molecule binding to the human CD40-Fc fusion protein was detected by FACS analysis on the surface of activated JF7 cells (Fig. 1B). RNA isolated from JF7 cells stimulated for 2 hours with ionomycin and PMA was therefore used to clone the human CD40-L cDNA. Poly A^+ RNA isolated from these cells (Fig. 1A) was subjected to reverse transcription and cloned in λgt10. One million plaques of the unamplified library were screened with the radio labelled *Bam*HI-*Hin*dII mouse CD40-L cDNA restriction fragment using hybridisation conditions deduced from the Northern blot assay (Fig. 1). 0.5–1% of the plaques gave a positive signal on both replica membranes subjected to the hybridisation, indicating that human CD40 mRNA represented a fairly abundant mRNA in stimulated JF7 cells. Ten independent λgt10 clones were isolated by multiple rounds of screening. Seven clones contained an insert which could be amplified by polymerase chain reaction (PCR) using λgt10 primers. Four clones were identical except for minor variations in the 5′ and 3′ ends, according to restriction mapping and sequencing. The DNA sequence of one of these inserts is represented in Fig. 2. Two inserts represented truncated cDNAs and the third comprised a CD40-L cDNA artefactually linked to another cDNA.

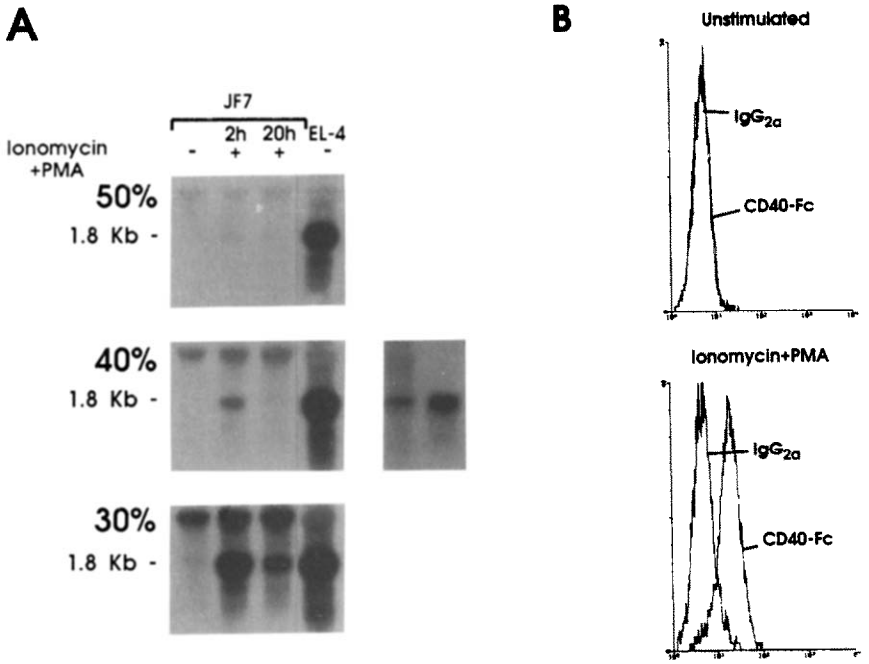

Fig. 1. Expression of human CD40-L mRNA (A) and surface protein (B) by JF7 cloned $CD4^+$ T cell clone as detected by cross-hybridisation using a mouse CD40-L cRNA probe and FITC-labelled CD40-Fc fusion protein, respectively. Northern blot assays were performed using 2 μg unfractionated or 0.3 μg poly A^+ RNA isolated from the human JF7 $CD4^+$ T cell clone or murine EL-4 cells unstimulated (−) or stimulated (+) for the times indicated with ionomycin (1 μM) and PMA (10 ng/ml). Decreasing concentrations of formamide (50%, 40%, 30%) were used to obtain cross-hybridisation between the murine cRNA probe and the RNA isolated from the human T cells. Autoradiography exposure time: 20 h. Conditions for the flow cytometry analysis of CD40-Fc or IgG_{2a} binding as described in section 2.

3.2. *Human CD40-Ligand sequence*

Sequencing of the λgt10 clone 6 revealed a 1.8 kb mRNA containing an open reading frame coding for 261 amino acids highly homologous to the mouse CD40-L cDNA coding region (77.4%; Fig. 3). The deduced amino acid sequence encodes for 13 amino-terminal amino acids, followed by a 32-amino acid stretch of hydrophobic residues which could represent the signal/anchor domain (Fig. 2). The protein contained two (one extracytoplasmic) potential glycosylation sites (Fig. 2). As shown for the mouse CD40-L [10], the putative transmembrane segment deduced from the hydrophobicity plot is followed by a potential protease cleavage site. The human CD40-L has five cysteines, compared to four for the murine CD40-L [10].

The human CD40-L shares homology with TNFα (23.4%) and TNFβ (20.7%), which has led to a predicted compact trimer structure (Peitsch, M.C. and Jongeneel, V.C., personal communication). The 3′ non-coding region of the CD40-L mRNA contains AUUU repeats which have been shown to confer instability to mRNA [26] as well as a long stretch of 33 CA repeats.

To show that the cloned cDNA was encoding for a protein able to bind CD40, BHK cells were transfected with a 1.1 kb *Eco*RI restriction sub fragment of clone 6 recloned in the pCDM8 expression vector in either sense or anti-sense orientation. Molecules binding to the human CD40-Fc fusion protein could clearly be detected at the surface of BHK cells transfected with the sense construct (Fig. 4).

3.3. *Expression of human CD40-Ligand mRNA and protein*

A radio labelled cRNA complementary to the *Eco*RI-*Hin*dIII fragment of human CD40-L cDNA was used to probe for the corresponding mRNA in human cell lines of various origins. Cells were probed either unstimulated or after 4 hours stimulation with ionomycin and PMA. Among the T cell lines HuT-78, MOLT-4 and Jurkat, B cell lines RPMI 8226, 8866, JY, BL-2 and IM-9 ; the histiocytic lymphoma cell line U-937 and the monocytic cell line THP-1, we detected the CD40-L mRNA only in Jurkat (Fig. 5A and data not shown). Surface expression of CD40-L by Jurkat was shown by immunofluorescence using CD40-Fc (Fig. 5B).

We studied CD40-L mRNA synthesis in PBMNC using the human cRNA probe. CD40-L mRNA was detected in unstimulated PBMNC (Fig. 6B). To exclude the possibility that this synthesis was a result of in vitro stimulation, RNA was isolated immediately after PBMNC isolation. CD40-L mRNA could clearly be detected in RNA from freshly isolated PBMNC (Fig. 6C). CD40-L mRNA is strongly upregulated by stimulation with ionomycin plus PMA (Fig. 6A), with a maximum steady state level at about 3 hours. Binding of FITC-labelled CD40-Fc paralleled the mRNA level and

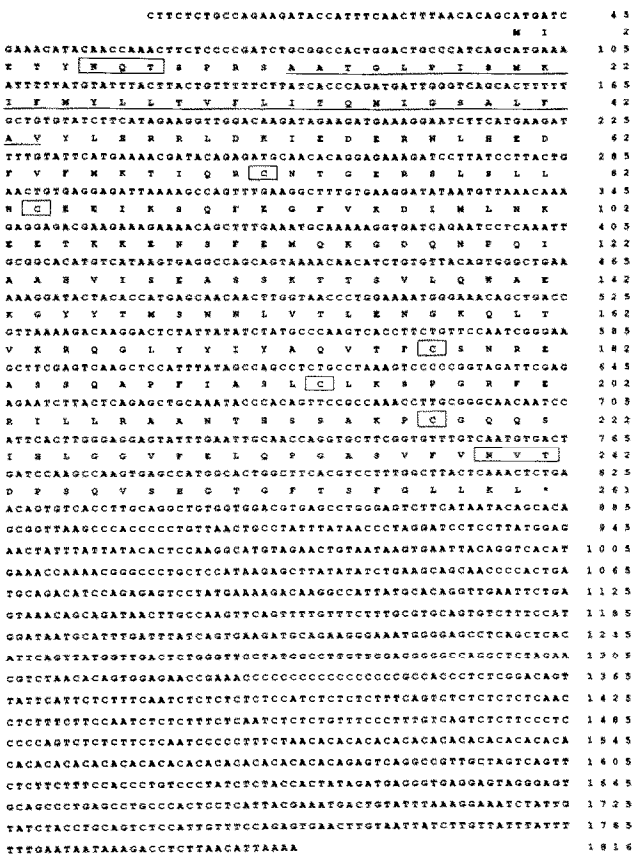

Fig. 2. Nucleotide and predicted amino acid sequence of the cDNA clone 6. The two potential *N*-glycosylation sites are boxed. The putative transmembrane domain is underlined and the five cysteines are boxed

showed that the expression of the ligand could be induced in a large fraction of the PBMNC (Fig. 6). To determine which immunocompetent cells could be induced to express CD40-L mRNA, cell populations positive for CD4, CD8, CD45R0, CD45RA, CD14 and CD20 were isolated from PBMNC by FACS and stimulated for 6 hours with ionomycin and PMA . RNA was tested for the presence of CD40-L mRNA by Northern blot. A signal could be detected only in stimulated and unstimulated CD4, CD8, CD45R0 and CD45RA cells (Fig. 7A and data not shown). Moreover $CD8^+/CD57^-$ and $CD8^+/CD57^+$ cells were purified by FACS sorting and stimulated for 6 hours with ionomycin and PMA. CD40-L mRNA was detected by Northern blot hybridisation on both activated cell populations (data not shown).

To confirm that both CD40-L mRNA and protein could be detected in CD8-positive cells we stimulated the $CD3^+$, $CD8^+$, $CD16^-$, clone 0ZZ 50. 1 with ionomycin plus PMA. A signal for the CD40-L mRNA could clearly be detected (Fig. 7A). Immunofluorescence using FITC-labelled CD40-Fc indicated that synthesis of the mRNA was paralleled by appearance of CD40-L at the surface of 20% of the cells (data not shown).

To show that CD40-L could be detected on the surface of activated $CD4^+$, $CD8^+$, $CD45R0^+$ and $CD45RA^+$ cells, PBMNC activated for 6 hours were double labelled using different anti-CD mAbs and CD40-Fc (Fig. 7B). Binding of CD40-Fc was restricted to $CD3^+$ cells with less than 1% of $CD3^-$ cells expressing CD40-L. The majority of the $CD4^+$ cells (79%) expressed the CD40-L as judged by CD40-Fc binding (Fig. 7). CD40-L could be detected on 41% of the acti-

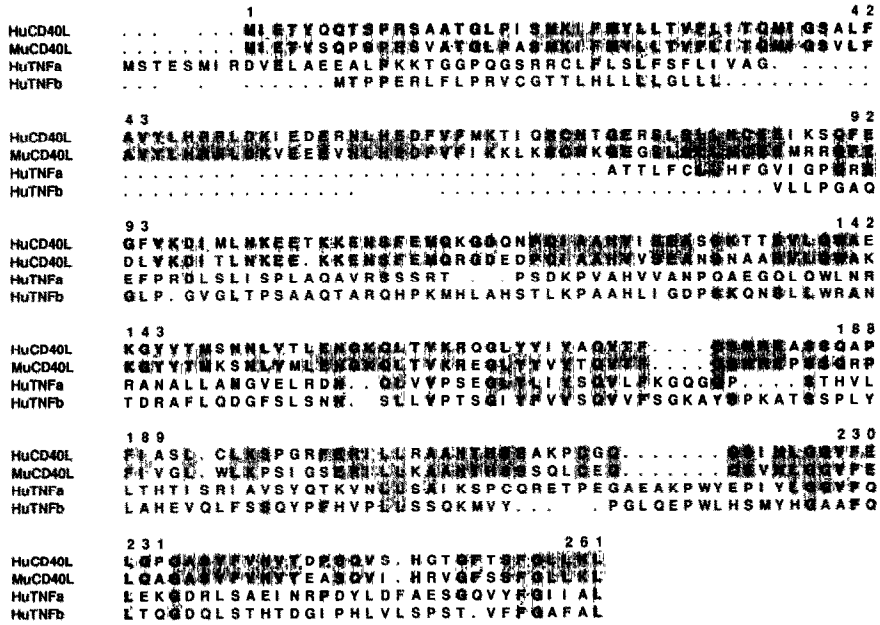

Fig. 3. Alignment of the primary sequence of human CD40-L with murine CD40-L, human TNFα and TNFβ . Areas of amino acid identity (dark) or similarity (shaded) are indicated.

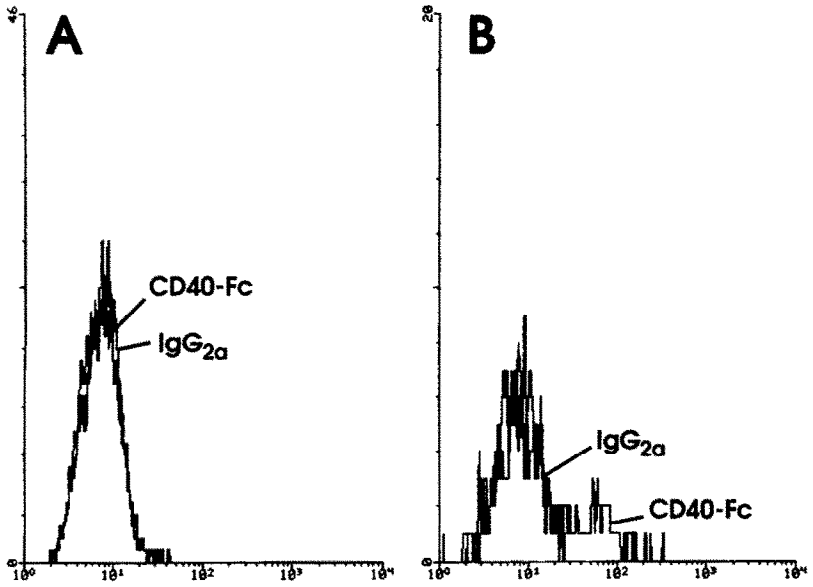

Fig. 4. Expression of human CD40-L in BHK cells transfected with CD40-L cDNA in anti sense (A) or sense orientation (B). Transfected BHK cells were analysed for CD40-Fc or IgG2a binding as indicated in Fig. 1.

vated $CD8^+$ cells (Fig. 7). We did not observe differences between 'memory' or 'virgin' T cells as expression of CD40-L was induced on 72% of both $CD45RA^+$ and $CD45R0^+$ cells (Fig. 7B). Under the activation conditions used, induction of CD40-L synthesis was restricted to T cells and could not be detected on B cells (< 0.3%), NK cells (< 0.8%) or monocytes (< 1%).

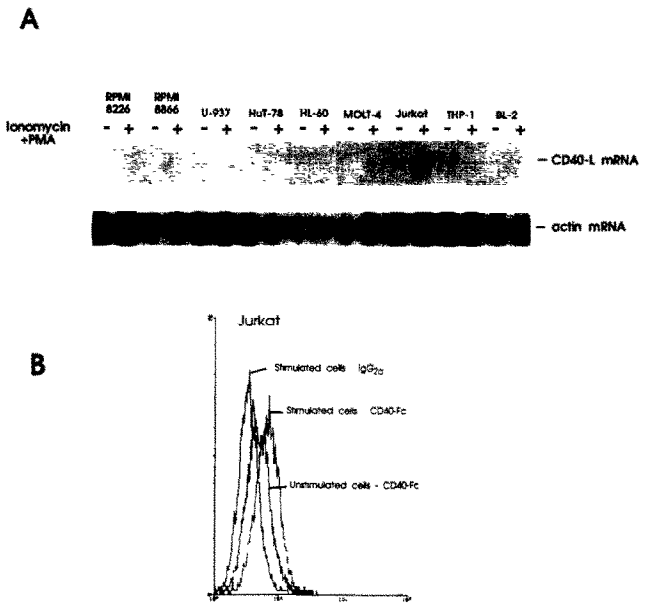

Fig. 5. CD40-L mRNA level in nine human cell lines (A) and CD40-L protein in Jurkat cells (B). RNA (2 μg aliquots) isolated from unstimulated cells (−) or cells incubated (+) for 4 h with ionomycin (1 μM) and PMA (10 ng/ml) were subjected to Northern blot assay with CD40-L cRNA (upper autoradiograph) or actin cDNA (lower autoradiograph) probes. Auroradiography exposure time: 72 h. Jurkat cells were analysed for CD40-Fc or IgG_{2a} binding as indicated in Fig. 1.

3.4. *Effect of IgE regulatory cytokines on CD40-Ligand mRNA level*

Murine CD40-L in conjunction with IL-4 has been shown to induce IgE synthesis in purified human B cells [10]. We observed that human CD40-L and its mRNA could both be detected in $CD4^+$ T cells clones 5 days after stimulation with irradiated PBMNC, PHA and IL-2 (Fig. 8 and data not shown). Human $CD4^+$ T cell clones activated using these conditions are capable of inducing, in the presence of IL-4, IgE synthesis by human B cells [27,28]. The expression of CD40-L mRNA was stable for 9 days in activated T cell clones cultured in the presence of IL-4 alone as illustrated for the clone JF7 (Fig. 8). This satisfied time required for switching to the ε constant region and IgE synthesis [27,28]. These findings indicate that synthesis of the CD40-L could be detected under conditions which are used to promote IgE synthesis in vitro.

IgE synthesis can be induced by IL-4 alone in PBMNC. We therefore studied if induction of IgE production was paralleled by a change in CD40-L synthesis. We observed that incubation of PBMNC with IL-4 more than 6 days reproducibly caused up regulation of CD40-L mRNA as illustrated in Fig. 8. Immunofluorescence using CD40-Fc was not sensitive enough to detect CD40-L after stimulation with IL-4 alone. In contrast, the up regulation of CD40-L mRNA was prevented by the addition of IFNγ (Fig. 8). Together these findings indicate that CD40-L expression is influenced by factors which regulate IgE production.

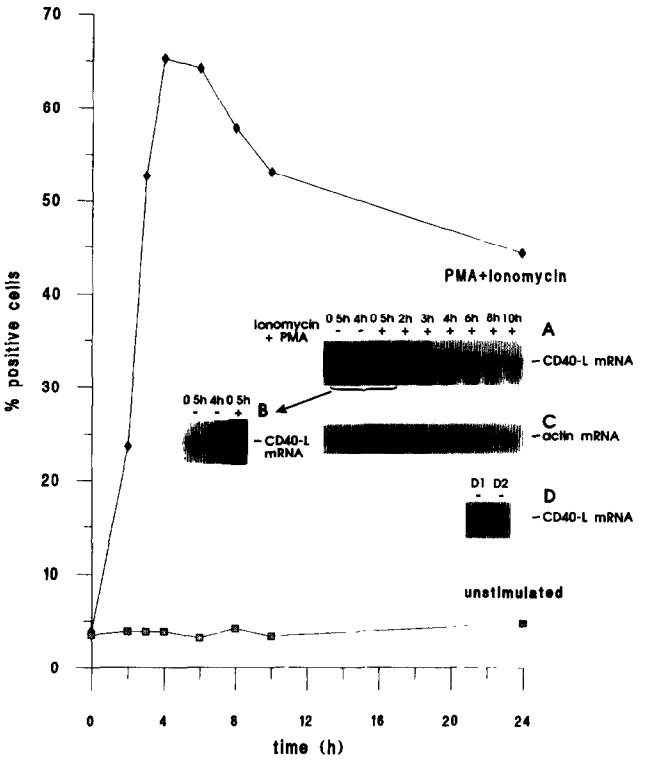

Fig. 6. CD40-L protein and CD40-L mRNA expression in PBMNC immediately after isolation (D; D1 and D2 represents two different blood donors) or PBMNC cultured for the indicated times in the absence or presence of ionomycin (1 μM) and PMA (10 ng/ml). Northern blot and binding assays were performed as described in Figs. 4 and 1. Autoradiography exposure time: A, 1 h; B, C and D, 20 h.

4. DISCUSSION

We report here the molecular cloning of the human CD40-ligand cDNA. Human and murine CD40-L share a significant protein sequence homology (77.4%) which is consistent with the functional cross-reactivity observed recently by Armitage et al. [10]. The encoded polypeptide of 261 amino acids consists of 13 amino-terminal amino acids, followed by a 32-amino acid sequence of hydrophobic residues, which may function as a signal/anchor domain. This configuration is typical for a type-II transmembrane protein in which the carboxy terminus is extracellular. The human CD40-L differs from the murine counterpart in that it contains two potential N-glycosylation sites instead of one (one being intracellular), five cysteins instead of four and one extra amino acid in position 105. The homology of murine CD40-L with TNFα and TNFβ , which is shared by human CD40-L, has led to a predicted trimer structure (Peitsch, M.C. and Jongeneel, V.C., personal communication).

Transfection of the human CD40-L cDNA recloned in pCDM8 [21] was followed by detectable binding to CD40-Fc indicating that the cDNA could encode for a functional protein.

We studied CD40-L mRNA and protein synthesis in PBMNC. The CD40-L mRNA could be detected in unstimulated cells. Stimulation of PBMNC with ionomycin and PMA is followed by a very strong increase in the CD40-L mRNA level, which is detectable at 30 minutes. This is accompanied by the ability of a majority of cells to bind CD40-Fc fusion protein as recently reported [29]. Studies on PBMNC subpopulations using Northern blot assays or double labelling and FACS analysis indicated that synthesis of the CD40-L mRNA and protein could be induced on a fraction of CD4, CD8, CD45RA, CD45R0 but not CD20 or CD14 positive cells. The expression of the CD40-L mRNA and protein observed in CD8$^+$ cells was confirmed using a CD8$^+$ T cell clone. This finding is in line with the report

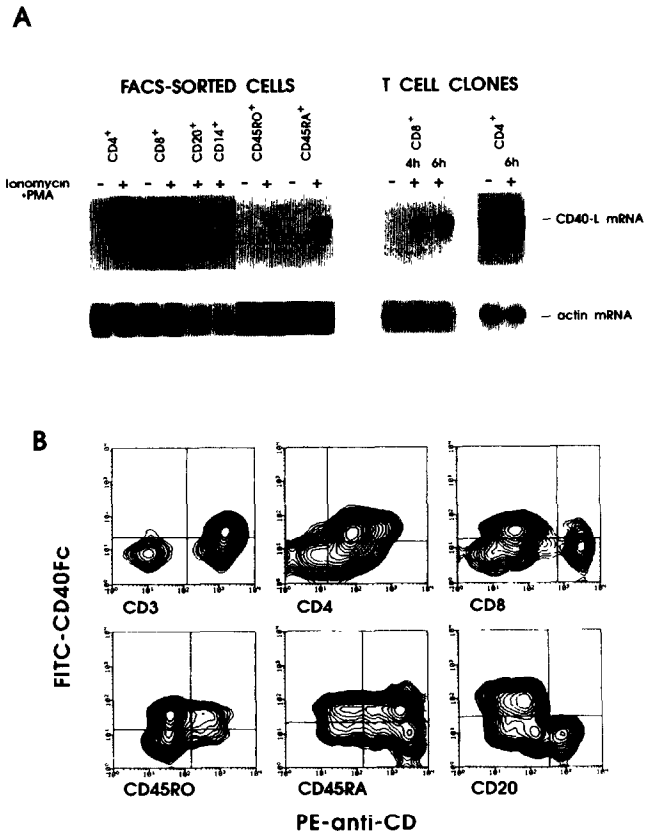

Fig. 7. Analysis of CD40-L mRNA level (A) or CD40-Fc binding (B) in PBMNC sub populations and in CD8$^+$ and CD4$^+$ T cell clones. (A) PBMNC sub populations were FACS sorted using mAbs directed against the indicated markers (see section 2). Cells (10^6/ml) were incubated for 4 h in medium alone (−) or in the presence (+) of ionomycin (1 μM) and PMA (10 ng/ml). RNA isolated from 8×10^6 (CD4$^+$), 3.5×10^6 (CD8$^+$), 5×10^6 (CD20$^+$), 6×10^6 (CD14$^+$), 3×10^6 (CD45RA$^+$), 2.5×10^6 (CD45 R0$^+$) cells was subjected to Northern blot analysis with CD40-L cRNA or actin cDNA probes. Parallel analysis was done with RNA from the indicated cloned T cells. Autoradiography exposure time: CD40-L probe, 2 h; actin probe, 48 h. (B): Contour plot display of PBMNC incubated with optimal concentrations of FITC-CD40-Fc and PE-labelled monoclonal antibodies directed against the indicated markers. FACS settings and electronic compensation were made on single population of labelled cells.

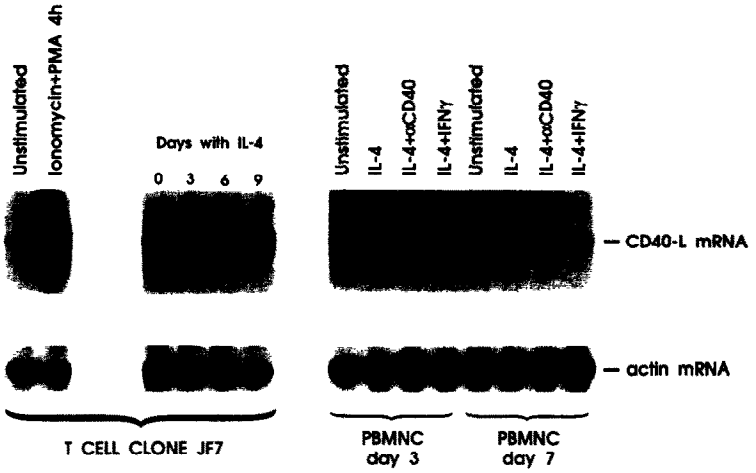

Fig. 8. Expression of CD40-L mRNA in cloned CD4$^+$ T cells and PBMNC activated under conditions used to promote IgE synthesis in vitro. Cloned JF7 cells were activated for 5 days with PHA (0.2 μg/ml), irradiated PBMNC 10^6/ml and IL-2 50 U/ml), washed and cultured for the times indicated with IL-4 alone (200 U/ml). As a control, resting (day 16 after last stimulation) JF7 cells were stimulated with ionomycin (1μM) and PMA (10 ng/ml). PBMNC were incubated for the times indicated with medium alone, IL-4 (200 U/ml), IL-4 and anti-CD40 mAb (1 μg/ml) or IL-4 and IFNγ (500 U/ml). Northern blot assays for CD40-L and actin mRNA were performed as described in Fig. 4. Autoradiography exposure time: 24 h.

that some CD8$^+$ T cell clones can be used in conjunction with IL-4 to trigger IgE synthesis [30].

Binding of CD40 by anti-CD40 mAbs or murine CD40-L was shown to induce, in the presence of IL-4, switching to the ε constant region as well as induction of IgE synthesis by purified human B cells [6–10]. In line with these observations, anti-CD40 mAbs induce in B cells a strong up regulation of the germline ε transcript synthesis triggered by IL-4 [9,31]. The second signal necessary to induce IgE synthesis in purified B cells can be given by cloned CD4$^+$ T cells activated for five days with PHA, irradiated PBMNC and IL-2 or membranes isolated from stimulated T cells [27,28]. These findings are in accordance with a current model in which two signals are required for IgE production (reviewed in [32]). The first signal is given by the soluble cytokine IL-4 [33] which induces germline ε transcript expression [27] but alone is insufficient to trigger secretion of IgE. The second signal is provided by a physical interaction between activated T and B cells (reviewed in [32]). The CD40/CD40-L pair appears therefore to be a candidate for mediating signalling between T and B cells, leading to IgE synthesis.

One aim of the present study was to determine whether CD40-L was expressed on immunocompetent peripheral blood cells and if expression varied under conditions known to affect IgE synthesis. Using a human CD40-L cRNA probe, it was possible to detect the corresponding mRNA in both unstimulated and stimulated CD4$^+$ and CD8$^+$ T cell clones. In CD4$^+$ cells, the mRNA level is very strongly upregulated shortly after stimulation with ionomycin plus PMA. The results of screening the λgt10 library made with RNA isolated from stimulated CD4$^+$ T cells, suggest that this mRNA represents a large fraction (0.5–1%) of the poly A$^+$ RNA. More relevant for the in vitro model used to study IgE synthesis, the CD40-L mRNA and the corresponding protein can clearly be detected five days after activation of cloned CD4$^+$ cells with PHA, irradiated PBMNC and IL-2. It is stable when IL-2 is replaced by IL-4.

We examined the expression of the CD40-L in PBMNC stimulated with IL-4 under conditions which trigger IgE synthesis. Interestingly, under such conditions the CD40-L mRNA level was upregulated. This was paralleled by an increase in CD40-Fc binding. This upregulation was prevented by IFNγ, a cytokine which inhibits IL-4-induced IgE production by PBMNC [34]. This indicates that it belongs to the category of IL-4-induced effects which can be blocked by IFNγ.

Taken together, these results indicate that the synthesis of CD40-L can be induced by stimulation of various T cell subtypes. The expression of both CD40-L mRNA and protein is also observed under conditions which are used to trigger switching to the ε constant region and also IgE synthesis. This suggests that the CD40-L provides an important signal in the induction of IgE synthesis in vitro. The possibility that it represent an adhesion-type of surface molecules involved in T cell-B cell interaction such as the CD23 / CD21 pair [35] is under investigation.

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