CD30-mediated Signaling Promotes the Development of Human T Helper Type 2-like T Cells

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Summary

We have recently shown that CD30, a member of the tumor necrosis factor/nerve growth factor receptor superfamily, is preferentially expressed by human T cell clones producing T helper (Th) type 2 cytokines. We report here that costimulation with an agonistic anti-CD30 monoclonal antibody enhanced antigen (Ag)-induced proliferation and cytokine secretion by established human Th2 and Th0 clones. Moreover, costimulation of peripheral blood mononuclear cells with the same anti-CD30 monoclonal antibody resulted in the preferential development of Ag-specific T cell lines and clones showing a Th2-like profile of cytokine secretion. In contrast, early blockade in bulk culture of CD30 ligand–CD30 interaction shifted the development of Ag-specific T cells towards the opposite (Th1-like) phenotype. Taken together, these data suggest that CD30 triggering of activated Th cells by CD30 ligand–expressing Ag-presenting cells may represent an important costimulatory signaling for the development of Th2-type responses.

C D4+ T cells can be separated both functionally and based on the cytokines they produce in response to Ag (1). Th1 cells secrete IL-2, TNF-β, and IFN-γ, and, because of the macrophage-activating property of IFN-γ, they are the principal effectors of cell-mediated immunity against intracellular microbes as well as of delayed-type hypersensitivity reactions (2, 3). However, Th2 cells produce IL-4, IL-5, and IL-10, which promote production of antibodies (including IgE), differentiation, and activation of eosinophils, and inhibit several macrophage functions. Th2 cells mainly develop in response to helminths as well as to common environmental allergens (4, 5).

Several studies in both murine and human models have clearly shown that the development of both naive and memory CD4+ T cells into the Th1 or Th2 functional phenotype is mainly regulated by cytokines (6, 7). IL-4 induces Th to differentiate into IL-4–producing cells and diminishes their priming for IFN-γ production, thus polarizing the response toward the Th2 state (7, 8); IL-12 strikingly enhances priming for IFN-γ production, and, through the production of IFN-γ by T cells and NK cells, IL-12 diminishes priming for IL-4 production, thus polarizing the response toward a Th1 state (9, 10). However, these findings do not fully and directly explain which features of a natural or intentional immunization elicit the distinctive production of the inducing cytokines or other possible factors that may determine the phenotype of cytokine-producing Th cells.

Recently, we have shown that CD30, a member of TNF

receptor superfamily (11), is preferentially expressed by Th2-type CD4+ T cell clones (12) as well as by CD8+ T cell clones showing a Th2-like profile of cytokine secretion (13). The natural ligand for CD30 (CD30L)[1] has been cloned (14), and it has been found to be expressed by B lymphocytes (15) as well as by a subset of activated macrophages and T cells (16). We have therefore addressed the question of whether costimulation of CD30 had any functional role in the Ag-induced response of established Th2 clones as well as in the in vitro development and/or maturation of Th2 cells.

Results showed that CD30 costimulation potentiated both Ag-induced proliferative response and cytokine production by established Th2 and Th0 clones. More importantly, CD30 costimulation favored the in vitro development of Ag-specific T cells toward the Th2-like profile, whereas blockade of CD30L–CD30 interaction had an inhibitory effect on the development of Ag-specific memory T cells into the Th2-like phenotype. Thus, CD30 triggering on Ag-reactive T cells by APC expressing CD30L may provide a critical costimulatory signal for the development of Th2 responses.

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[1] Abbreviations used in this paper: CD30-Fc, soluble form of CD30 fused to the Fc region of human IgG1; CD30L, natural ligand for CD30; Lol p I, Lolium perenne group I; TES, excretory–secretory Ag of Toxocara canis; TT, tetanus toxoid.

Materials and Methods

Reagents. Excretory–secretory Ag of *Toxocara canis* (TES) was purchased by Biokema (Lausanne, Switzerland), tetanus toxoid (TT) was kindly provided by Istituto Sieroterapico e Vaccinogeno Sclavo (Siena, Italy), and purified *Lolium perenne* group 1 (Lol p I) allergen was a gift from Dr. A. A. Ansari (Johns Hopkins University, Baltimore, MD). Recombinant human IL-2 was a kind gift from Eurocetus (Milano, Italy), and recombinant human IL-5 was purchased from Amgen Biologicals (Thousand Oaks, CA). PHA was purchased from Gibco Laboratories (Grand Island, NY). Anti-CD3, anti-CD4, and anti-CD8 mAb were purchased from Ortho Pharmaceuticals (Raritan, NJ). Anti-CD30 mAb (Ber-H2) was purchased from Dako (Glostrup, Denmark). Anti-CD30 agonist M44 mAb, anti-CD30L M81 mAb, and the soluble form of CD30 fused to the Fc region of human IgG1 (CD30-Fc) were prepared by Immunex (Seattle, WA). Polyclonal rabbit anti–human IL-4 was purchased from Genzyme Corp. (Cambridge, MA).

Generation of T Cell Lines and Clones. PBMC were obtained either from healthy volunteers who had been vaccinated with TT and showed a lymphoproliferative response in vitro to TES or from atopic grass-sensitive subjects showing immediate skin reactivity to Lol p I allergen. Ag-specific T cell lines were generated as previously described (17). Briefly, PBMC prepared by density gradient centrifugation were resuspended (10^6 in 2 ml) in RPMI 1640 medium supplemented with 2 mM L-glutamine, 2×10^{-5} M 2-ME, and 5% heat-inactivated human serum (complete medium) and stimulated with TES, Lol p I (2 μg/ml), or TT (0.5 μg/ml) in 24-well plates for 6 d in the presence of immobilized anti-CD30 agonist M44 mAb (10 μg/ml) or isotype control (murine IgG1), soluble anti-CD30L M81 mAb (5 μg/ml) or its isotype control (murine IgG2b), and soluble CD30-Fc (1 μg/ml) or its control (human IgG1). After 6 d IL-2 was added (20 U/ml), and cultures were continued for an additional 5 d. Ag specificity was assessed by measuring [^{3}H]TdR uptake after 60 h of stimulation with the appropriate Ag in the presence of irradiated autologous non–T cells as APC, as reported (17). To generate T cell clones, T cell blasts obtained from the different T cell lines were seeded under limiting dilution conditions (0.3 cells per well) in round-bottomed microwell plates containing 10^5 irradiated PBMC (as feeder cells), PHA (0.5% vol/vol), and IL-2 (20 U/ml), as reported elsewhere in detail (17). Cell surface marker analysis of T cell clones was performed on a cytofluorimeter (Cytoron Absolute; Ortho Pharmaceuticals), and Ag specificity was assessed by measuring [^{3}H]TdR uptake after 60 h of stimulation with the appropriate Ag, as reported (17).

Characterization of the Cytokine Profile of T Cell Lines and Clones. To induce cytokine production by T cell lines or clones in response to their specific Ag, 10^6 T cell blasts were cocultured in 1 ml complete medium with 5×10^5 irradiated autologous APC and the specific Ag (0.5–2 μg/ml) (17). After 48 h, culture supernatants were collected, filtered, and stored in aliquots at $-70°C$ until used. To induce cytokine production in response to PHA, T cell blasts were resuspended at 10^6/ml complete medium and cultured in the presence of PHA (1% vol/vol). After 36 h, culture supernatants were collected, filtered, and stored in aliquots at $-70°C$ until used. Supernatants were assayed for IFN-γ, TNF-β, IL-4, and IL-5 content. The quantitative determinations of IFN-γ or TNF-β and IL-4 were performed by commercial assays (BioSource International, Camarillo, CA; or Quantikine, R&D Systems, Minneapolis, MN, respectively). For the measurement of IL-5, the murine LyH7.B13 cell line was used as source of indicator cells, as detailed elsewhere (17). Supernatants showing IFN-γ, TNF-β, IL-4, or IL-5 levels 5 SD over the mean levels in control

supernatants derived from irradiated feeder cells alone were regarded as positive. T cell clones able to produce IFN-γ and TNF-β, but not IL-4 or IL-5, were categorized as Th1; clones able to produce IL-4 and/or IL-5, but not IFN-γ and/or TNF-β, were categorized as Th2; and clones producing both IFN-γ (and/or TNF-β) and IL-4 or IL-5 were categorized as Th0.

Results

CD30 Costimulation Enhances Ag-induced Proliferation and Cytokine Production by Established CD4⁺CD30⁺ T Cell Clones. We first investigated the responsiveness of a panel of CD30⁺ and CD30⁻ CD4⁺ T cell clones either to stimulation with an anti-CD30 agonist (M44) mAb or to costimulation with M44 mAb and the specific Ag. To this end, six Th1, six Th0, and six Th2 clones (whose mean CD30 expression was <10, 14–56, and 41–87%, respectively) were cultured for 3 d in the presence of immobilized M44 alone or M44 plus Ag-primed autologous APC, and their proliferative response was assessed. No response to M44 mAb was seen in the absence of Ag-primed APC stimulation (data not shown); however, Ag-driven proliferation was significantly increased by M44 mAb ($P < 0.01$) in both Th0 and Th2 (CD30⁺) but not in Th1 (CD30⁻) T cell clones (Table 1). To ascertain whether the costimulatory activity of immobilized anti-CD30 might be ascribed to CD30 cross-linking, Ag-induced proliferation of two Th1, two Th0, and two Th2 clones was assessed in microplates coated with graded concentrations of M44 mAb or in the presence of soluble M44 followed by a polyclonal rabbit anti–mouse Ig Ab. As shown in Table 2, the

Table 1. *Costimulatory Effect of Immobilized Anti-CD30 Agonist M44 mAb on Antigen-induced Proliferation of Th0 and Th2 T Cell Clones*

T cell clones	Costimulant	Mean (± SD) [^{3}H]thymidine uptake	Mean (± SD) % increase
		cpm $\times 10^{-3}$	
Th1	Isotype control	9.5 ± 2.3	
	M.44	10.6 ± 2.7	10.4 ± 11
Th0	Isotype control	11.2 ± 4.5	
	M.44	14.5 ± 5.1	29.5 ± 13
Th2	Isotype control	9.3 ± 3.8	
	M.44	16.1 ± 6.6	73.1 ± 28

T cell clones specific for TT, Lol p I, or TES were stimulated for 36 h with PHA, and cell-free supernatants were assayed for their IL-4, IL-5, and IFN-γ content as reported (17). Six Th1, six Th0, and six Th2 CD4⁺ clones, whose T cell blasts showed membrane CD30 expression of <10, from 14 to 56, and from 41 to 87%, respectively, were costimulated with insoluble M44 (10 μg/ml) or its isotype control in the presence of autologous APC and the specific Ag (TT, 0.5 μg/ml; TES or Lol p I, 2 μg/ml) as described. After 3 d, [^{3}H]thymidine uptake was evaluated. Results express mean values (± SD) of triplicate cultures for each series of six clones.

Table 2. *Costimulatory Activity of Anti-CD30 Agonistic M.44 mAb Is Due to CD30 Cross-linking*

Culture condition	Antigen-induced [³H]thymidine uptake (cpm × 10⁻³)			
	T.61 (Th0)	T.22 (Th0)	T.36 (Th2)	T.42 (Th2)
Insoluble control Ig (50 μg/ml)	8.61	12.11	8.90	12.98
Insoluble M.44 (10 μg/ml)	11.15 (37)	16.32 (30)	15.81 (75)	23.72 (81)
Insoluble M.44 (2 μg/ml)	10.65 (25)	15.03 (22)	14.21 (61)	20.66 (62)
Insoluble M.44 (0.4 μg/ml)	9.17 (8)	13.85 (8)	10.92 (20)	16.19 (26)
Insoluble M.44 (10 μg/ml) + CD30-Fc (5 μg/ml)	8.32 (3)	13.12 (5)	11.75 (30)	17.12 (31)
Soluble control Ig (5 μg/ml) + anti–mouse Ig (50 μg/ml)	8.36	12.02	9.07	12.47
Soluble M.44 (5 μg/ml) + anti–mouse Ig (50 μg/ml)	12.09 (45)	15.89 (32)	16.13 (78)	21.70 (74)

Four TES-specific clones (two Th0 and two Th2), whose T cell blasts showed membrane CD30 expression of 19, 32, 56, and 67%, respectively, were stimulated with TES in the presence of graded concentrations of insoluble M.44 or its isotype control with or without addition of soluble CD30-Fc. Clones were also costimulated with soluble M.44 or control Ig, followed by a polyclonal rabbit anti–mouse Ig antibody. After 3 d, [³H]thymidine uptake was evaluated. Results express mean values of triplicate cultures. Values in parentheses indicate the mean percent increase of proliferation in comparison with the appropriate controls.

costimulatory effect of immobilized M44 mAb on Ag-induced proliferation of both Th0 and Th2 clones was dose dependent and was substantially inhibited by the addition of CD30-Fc. In the same experiment, costimulation of Ag-induced proliferation was also induced by soluble M44 followed by a polyclonal rabbit anti–mouse Ig Ab, suggesting that the activity of the agonist anti-CD30 mAb could be ascribed to CD30 cross-linking. We then asked whether costimulation of T cell clones with insoluble M44 mAb could also influence their ability to produce cytokines. To answer this question, IFN-γ, IL-4, and IL-5 production was measured in culture supernatants of the same Th1, Th0, and Th2 clones cultured for 48 h in the presence of M44 mAb (or its isotype control), with or without the addition of Ag-primed APC. Stimulation with M44 mAb alone did not result in the production of any detectable cytokine, whereas costimulation with M44 mAb, but not with isotype control, significantly increased both IL-4 and IL-5 Ag-induced production in all Th2 clones (P <0.001) and, to an even lower extent, in all Th0 clones (P <0.05). Under the same conditions, M44 mAb also significantly enhanced Ag-induced IFN-γ production by Th0 clones (P <0.05), but it had virtually no effect on IFN-γ production by Th1 clones (Fig. 1). No qualitative changes in the original cytokine profile of established T cell clones were observed, inasmuch as Th1 clones were not induced to secrete IL-4 or IL-5, nor were Th2 clones induced to secrete IFN-γ.

CD30 Triggering Favors the Preferential Expansion In Vitro of T Cells Producing Th2-Type Cytokines. The possibility that agonistic signals delivered through CD30 might induce the preferential expansion of Th cells producing Th2-type cytokines was then investigated. To this end, TT-specific T cell clones were generated from three different donors by

stimulating their PBMC with specific Ag in the absence or presence of insolubilized M44 mAb or of its isotype control. As shown in Fig. 2 A, in the series of 108 CD4⁺ T cell clones derived from M44 mAb-costimulated lines, the proportion of clones able to produce Th2-type cytokines (IL-4 and IL-5) was significantly increased, whereas the propor-
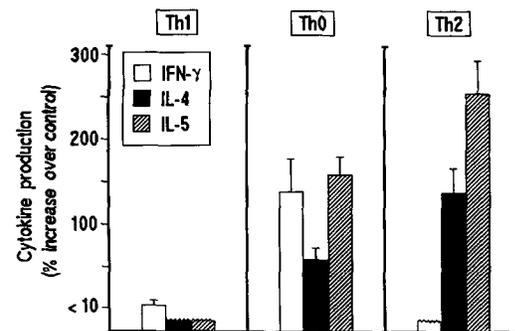

Figure 1. Costimulatory effect of immobilized anti-CD30 agonist M44 mAb on Ag-induced cytokine production by six Th1, six Th0, and six Th2 established T cell clones. To induce cytokine production, 10⁶ T cell blasts from each clone were seeded in tubes coated with M44 mAb (10 μg/ml) or its isotype control (murine IgG1) and cultured in 1 ml in the presence of 5 × 10⁵ irradiated autologous APC and the specific Ag (TT, 0.5 μg/ml; Lol p I or TES, 2 μg/ml). After 48 h, culture supernatants were collected, filtered, and assessed for cytokine content. Results represent the mean (± SD) percent increase of cytokine production over values measured in supernatants from the same T cell clones cultured in tubes coated with isotype control. Values of cytokine production were 68–232 U/ml IFN-γ, <30 pg/ml IL-4, and <0.2 U/ml IL-5 in control cultures of Th1 clones; 52–208 U/ml IFN-γ, 120–3,600 pg/ml IL-4, and 0.9–23.0 U/ml IL-5 in control cultures of Th0 clones; <3 U/ml IFN-γ, 90–4,800 pg/ml IL-4, and 3.2–36.5 U/ml IL-5 in control cultures of Th2 clones.
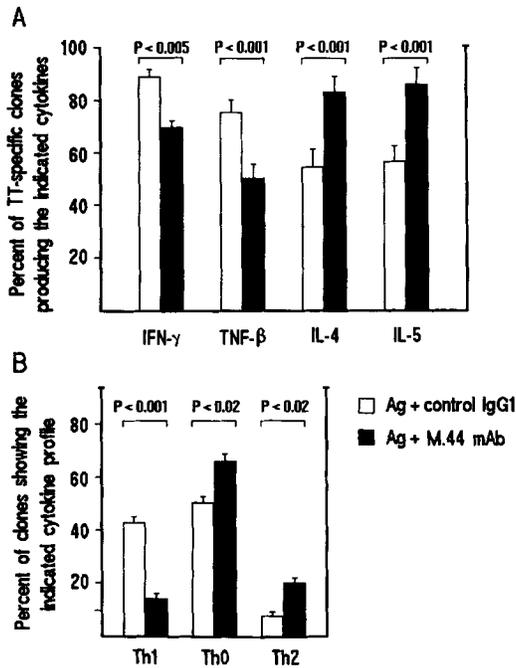
A

B

Figure 2. Costimulation with anti-CD30 agonist M44 mAb favors the preferential expansion of T cells producing Th2-type cytokines. Two series of TT-specific T cell lines were raised in the presence of insoluble M44 or its isotype control, as described in Table 1. On day 11, T cell blasts were cloned (0.3 cells per well) in the presence of PHA (1% vol/vol) and IL-2 (20 U/ml). A total number of 108 TT-specific CD4$^+$ clones derived from M44-costimulated T cell lines were compared with 90 TT-specific CD4$^+$ clones derived from isotype control–costimulated lines for their ability to produce IFN-γ, TNF-β, IL-4, and IL-5 upon 36 h of stimulation with PHA (*A*). The two series of TT-specific clones were also compared for their distribution of Th profiles (*B*). Results represent mean percent values ± SE. Statistical analysis was performed with the $\chi2$ test.

tion of clones producing Th1-type cytokines (IFN-γ and/or TNF-β) was significantly reduced in comparison with those found in the 90 clones derived from TT-specific lines costimulated with isotype control. When the two series of TT-specific clones were compared for the distribution of cytokine profiles, a significant reduction of Th1 (14 vs 43%) and an increase of both Th0 (67 vs 50%) and Th2 (19 vs 7%) phenotypes were found in the series costimulated with M44 mAb (Fig. 2 *B*). These data suggest that costimulation via CD30 favored the preferential development and/or the selective growth of Th cells able to produce Th2-type cytokines.

Blockade of CD30–CD30L Interaction Results in the Outgrowth of Ag-specific T Cell Lines and Clones Able to Produce Th1-Type Cytokines and Inhibits the Development of Th2-like T Cells. In view of the effects of anti-CD30 agonist mAb, we asked whether blockade of the interaction between CD30 and its natural ligand (CD30L) could also influence the cytokine secretion profile of Ag-specific T cells. To this end, Lol p I–specific T cell clones were generated from three different donors by stimulating their PBMC with Lol p I alone or Lol p I plus anti-CD30L mAb (M81), CD30-Fc fusion protein, or anti–IL-4 Ab. As expected, among

the 84 CD4$^+$ T cell clones generated in presence of anti–IL-4 Ab, the proportion of those showing a Th1-like profile was remarkably increased with concomitant reduction of Th2-like clones in comparison with the 86 CD4$^+$ T cell clones derived from Lol p I stimulated in presence of nonimmune rabbit Ig (Fig. 3). Likewise, either in the series of 101 CD4$^+$ T cell clones derived from anti-CD30L mAb–conditioned cells or in the series of 99 clones derived from CD30-Fc–treated cells, the proportions of clones able to produce IL-4 and IL-5 were significantly reduced, whereas the proportion of clones producing IFN-γ was significantly increased in comparison with that found in the 86 Lol p I–specific control clones. When the different series of clones were compared for their distribution of cytokine profiles, a significant increase of Th1 (34 and 31 vs 15%) and a decrease of Th2 (29 and 37 vs 58%) phenotypes were found in the series derived from cells conditioned with either anti-CD30L mAb or CD30-Fc (Fig. 3).

We finally asked whether blockade of CD30L–CD30 interaction was effective only in the early phases of T cell activation by Ag, or whether it could still influence the cytokine secretion profile of Ag-specific T cells if applied later. To this end, the effects of anti-CD30L M81 mAb and CD30-Fc were assessed in kinetics experiments in which the two reagents were added at different time intervals (day 0, 3, and 6) to PBMC cultures of seven donors stimulated with TES, TT, or Lol p I Ags. As expected, early blockade of CD30L–CD30 interaction by either M81 mAb or CD30-Fc fusion protein induced the outgrowth of Ag-specific T
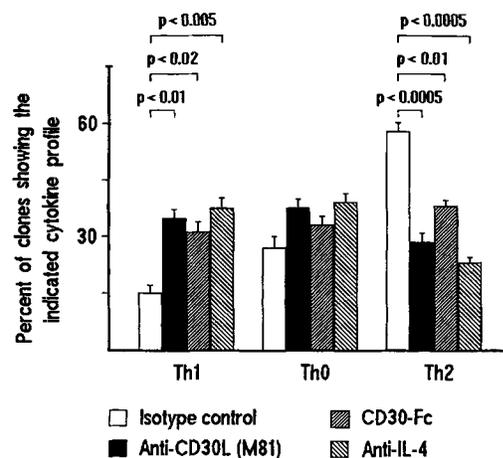

Figure 3. Blocking of CD30–CD30L interaction hampers the development of Ag-specific T cell clones into Th2-like cells. PBMC from three grass pollen–sensitive donors were stimulated with Lol p I Ag in the presence of anti-CD30L M81 mAb (5 μg/ml), pooled murine and rabbit control Ig (10 μg/ml), CD30-Fc (1 μg/ml), or rabbit polyclonal anti–IL-4 Ab (10 μg/ml). T cell blasts from each T cell line were cloned as detailed in Table 1. A total of 101 Lol p I–specific CD4$^+$ clones generated in the presence of M81 mAb, 86 Lol p I–specific CD4$^+$ clones generated in the presence of control Ig, 99 Lol p I–specific CD4$^+$ clones generated in the presence of CD30-Fc, and 84 Lol p I–specific CD4$^+$ clones generated in the presence of anti–IL-4 Ab were compared for their Th1, Th0, or Th2 profiles. Results represent mean percent values ± SE. Statistical analysis was performed with the $\chi2$ test.
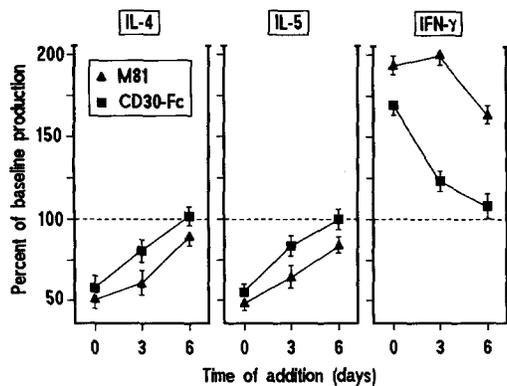
Figure 4. Kinetics of the effects of blocking of CD30–CD30L interaction by anti-CD30L mAb or CD30-Fc fusion protein. PBMC (10^6/ml) from five donors were stimulated with TES, TT, or Lol p I Ags in the presence of anti-CD30L M81 mAb (5 μg/ml), its isotype control, or CD30-Fc (1 μg/ml), added at different time intervals (day 0, 3, and 6). On day 6, IL-2 (20 U/ml) was added, and the cultures were continued for an additional 5 d. At the end of culture period, T cell lines were recovered and resuspended (10^6/ml) in fresh medium supplemented with PHA. After 36 h, supernatants were assayed for IL-4, IL-5, and IFN-γ content. Results represent mean ± SD percent increase/decrease of cytokine production versus values measured in supernatants from the corresponding T cell lines cultured in the presence of isotype control.

cell lines producing lower amounts of IL-4 and IL-5, but higher amounts (approximately a twofold increase) of IFN-γ than did their corresponding control T cell lines (Fig. 4). In contrast, when addition of the two CD30L-binding reagents was delayed to day 6 after Ag stimulation, T cell lines showed an IL-4 and IL-5 production almost comparable to that exhibited by their control lines. The same effect was seen for IFN-γ production in Ag-specific T cell lines induced with late addition of CD30-Fc. However, blocking of CD30L by anti-CD30L mAb, even if delayed until day 6, was still effective in inducing Ag-specific T cell lines able to secrete higher amounts of IFN-γ than did the corresponding control lines (Fig. 4).

Discussion

The results of this study indicate that CD30 plays an important role in both functional activity and development of Th2-like human CD4$^+$ T cells. CD30, originally described as a marker of Hodgkin's and Reed-Sternberg cells of Hodgkin's lymphoma (18) and as an activation molecule of lymphoid cells that are detectable in scanty number around the lymphoid follicles in lymphoid tissues (19), has been recently identified as a cytokine receptor belonging to the TNF receptor superfamily (11). CD30 exists as membrane glycoprotein chains of 105 and 120 kD, derived from a 90-kD precursor and a 57-kD intracellular form (20). The extracellular form of CD30 is proteolytically cleaved to produce an 88-kD soluble form of the molecule, which is released by CD30-expressing cells in vitro and in vivo (21). In short-term cultures, CD30 expression is inducible on T and B cells transformed by virus (e.g., by human T cell lymphotropic virus types 1 and 2 or Epstein-Barr virus)

(22) and on a small proportion of anti-CD3–stimulated CD45RO$^+$ (memory) T cells, which exhibit enhanced B cell helper activity and produce both IFN-γ and IL-5, thus resembling Th0 cells (23, 24). We have recently shown that, at the clonal level, membrane CD30 is preferentially expressed by human T cells producing Th2-type cytokines, e.g., Th2- and Th0-like cells (12, 25). The discrepancy between our findings and those reported by Alzona et al. (24, 26) is only apparent and probably relates to the differences in the experimental approach used. It is likely that the cells expressing CD30 in response to the short-term and APC-independent polyclonal stimulation used by Alzona et al. represent precursors of T cells that predominantly develop into Th0 cells. In their system, indeed, CD30$^+$ T cells seem to be Th0-like cells able to provide optimal B helper cell activity (24), but still lacking IL-4 production. In contrast, a clearer separation of Th1, Th0, and Th2 cells can be achieved in our model of Ag-specific T cell lines and clones, where repeated T cell stimulation in the presence of autologous APC makes IL-4 secretion easily detectable. The demonstration that CD30 triggering by anti-CD30 agonist Ab enhances Ag-induced proliferation and cytokine production by Th2 and Th0 but not by Th1 clones strongly supports our previous findings (12, 25). Thus, it appears that CD30 is not only preferentially expressed on Th cells producing Th2-type cytokines, but it also acts as a costimulatory molecule whose cross-linking is able to promote both expansion and effector function of Th2-like cells.

The most interesting observation emerging from this study, however, is that, at least in vitro, CD30 costimulation also appears to play an important role in the development of Th2-like cells. First, the addition in bulk culture before cloning of agonist anti-CD30 Ab resulted in the development of higher proportions of Th2-like T cell clones than those obtained in parallel cultures stimulated with Ag and isotype control. More importantly, the blockade of CD30L–CD30 interaction by addition in bulk culture of anti-CD30L Ab or a soluble CD30-Fc fusion protein favored the development of higher numbers of Ag-specific Th cells showing the opposite (Th1-like) phenotype. Whereas the enrichment of Th2 cells induced by costimulation with anti-CD30 Ab may simply be due to a selection process that neglects Th1 cells, the shift to the Th1 profile resulting from blockade of CD30L–CD30 interaction more likely reflects a true change in the differentiation process of Ag-stimulated Th cells growing in culture. Only early blockade of CD30L–CD30 interaction was indeed effective in inhibiting the development of a prevalent Th2 phenotype, whereas the later addition of either anti-CD30L Ab or soluble CD30-Fc had poor or no shifting effect. Thus, it is reasonable to suggest that the interaction of Ag-activated memory T cells expressing CD30 with CD30L$^+$ APCs represents an effective costimulatory signal for their differentiation into T cell clones producing Th2-type cytokines. Whether different APC can differently express CD30L is still unclear. CD30L is expressed by a subset of LPS-activated macrophages (16) and by the majority of B lymphocytes (15); no data regarding CD30 expression on dendritic cells are available.

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The importance of costimulatory signals provided by APCs for optimal activation of T lymphocytes is well known. Several receptor–counterreceptor pairs mediate adhesion between APCs and T cells and deliver activating signals to T cells. The interaction of B7 expressed on macrophages and dendritic cells with its CD28 receptor on T cells represents the best characterized example of this costimulation (27, 28). The B7–CD28 interaction has recently been found to promote optimal proliferation of terminally differentiated Th1 clones in both mice and humans (29–33). More importantly, in *Leishmania major*-infected mice, in vivo blockade of the B7–CD28 interaction at the time of parasite inoculation had a profound suppressive effect on the maturation of Th2 but not Th1 cells, suggesting that important functional differences may underlie the costimulatory signals used by these two subsets of CD4$^+$ T cells (34). Accordingly, the blockade of CD30L–CD30 interaction might also interfere with a critical pathway required for in vitro Th2 development. This finding raises the question of whether such an interaction can inhibit the production of IL-12, which is considered the most important signal for the maturation of Th1 cells (9, 10), or whether, alternatively, it may favor the early production of IL-4, which is required for the differentiation of both naive (7) and memory (8) CD4$^+$ T cells

into mature Th2 effector populations. In this regard, it is of note that the blockade of CD30L–CD30 interaction was at least as effective in inhibiting Th2 development as the addition of an anti–IL-4 Ab. This suggests that CD30 triggering by CD30L-expressing APCs might contribute in some way to the early IL-4 production required for Th2 development. The kinetics of blockade of CD30-mediated effects may also be consistent with up-regulation of CD30 during T cell activation. Indeed, the addition of either anti–IL-4 or anti-CD30L Ab at the time of Ag stimulation resulted in a partial (between 31 and 40%) but consistent reduction of the proportion of CD30$^+$ T cell blasts, as assessed by FACS$^®$ analysis (data not shown). Although preliminary, these findings suggest the existence of a complex network of interactions among CD30L expression, early IL-4 production, and regulation of CD30 expression in activated T cells that deserves more detailed investigation. Whatever the mechanism triggered by blockade of CD30–CD30L interaction is, the fact that CD30-Fc, a recombinant and stable analogue of the natural soluble CD30, is as effective as an anti-CD30L Ab in hampering the development of Th2-like cells allows us to consider this molecule as a potential tool in therapeutic strategies aimed to down-regulate undesired Th2 responses in vivo.

References

1. Mosmann, T.R., and R.L. Coffman. 1989. Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv. Immunol.* 46:111–147.
2. Mosmann, T.R., and R.L. Coffman. 1989. Different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7:145–173.
3. Street, N.E., J.H. Schumacher, T.A.T. Fong, H. Bass, D.F. Fiorentino, A. Leverah, and T.R. Mosmann. 1990. Heterogeneity of mouse helper T cells: evidence from bulk cultures and limiting dilution cloning for precursors of Th1 and Th2 cells. *J. Immunol.* 144:1629–1639.
4. Sher, A., and R.L. Coffman. 1992. Regulation of immunity to parasites by T cells and T cell-derived cytokines. *Annu. Rev. Immunol.* 10:385–409.
5. Romagnani, S. 1992. Induction of Th1 and Th2 response: a key role for the "natural" immune response? *Immunol. Today.* 13:379–381.
6. Seder, R.A., W.E. Paul, M.M. Davis, and B. Fazekas de St. Groth. 1992. The presence of interleukin-4 during in vitro priming determines the lymphokine-producing potential of CD4$^+$ T cells from T cell receptor transgenic mice. *J. Exp.*

Med. 176:1091–1098.
7. Hsieh, C.-S., A.B. Heimberger, J.S. Gold, A. O'Garra, and K.S. Murphy. 1992. Differential regulation of T helper phenotype development by IL-4 and IL-10 in an αβ-transgenic system. *Proc. Natl. Acad. Sci. USA.* 89:6065–6069.
8. Parronchi, P., M. De Carli, R. Manetti, C. Simonelli, S. Sampognaro, M.-P. Piccinni, D. Macchia, E. Maggi, G. Del Prete, and S. Romagnani. 1992. IL-4 and IFN (α and γ) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. *J. Immunol.* 149:2977–2983.
9. Hsieh, C.-S., S.E. Macatonia, C.S. Tripp, S.F. Wolf, A. O'Garra, and K.M. Murphy. 1993. Development of Th1 CD4$^+$ T cells through IL-12 produced by Listeria-induced macrophages. *Science (Wash. DC).* 260:547–549.
10. Manetti, R., P. Parronchi, M.G. Giudizi, M.-P. Piccinni, E. Maggi, G. Trinchieri, and S. Romagnani. 1993. Natural killer stimulatory factor (interleukin-12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4–producing Th cells. *J. Exp. Med.* 177:1199–1204.

11. Smith, C., T. Davis, D. Anderson, L. Solam, M.P. Beckmann, R. Jerzy, S.K. Dower, D. Cosman, and R.G. Goodwin. 1990. A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. *Science (Wash. DC)*. 248:1019–1023.

12. Del Prete, G., M. De Carli, F. Almerigogna, C.K. Daniel, M.M. D'Elios, G. Zancuoghi, F. Vinante, G. Pizzolo, and S. Romagnani. 1995. Preferential expression of CD30 by human CD4⁺ T cells producing Th2-type cytokines. *FASEB J.* 9:81–86.

13. Manetti, R., F. Annunziato, R. Biagiotti, M.G. Giudizi, M.-P. Piccinni, L. Giannarini, S. Sampognaro, P. Parronchi, F. Vinante, G. Pizzolo, and S. Romagnani. 1994. CD30 expression by CD8⁺ T cells producing type 2 helper cytokines. Evidence for large numbers of CD8⁺CD30⁺ T cell clones in human immunodeficiency virus infection. *J. Exp. Med.* 180:2407–2412.

14. Smith, C.A., H.-J. Gruss, T. Davis, D. Anderson, T. Farrah, E. Baker, G.R. Sutherland, C.I. Brannan, N.G. Copeland, N.A. Jenkins, et al. 1993. CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. *Cell.* 73:1349–1360.

15. Younes, A., S. Jaing, H. Gruss, and M. Andreeff. 1994. Expression of the CD30 ligand (CD30L) on human peripheral blood lymphocytes from normal donors and patients with lymphoid malignancies. *Blood.* 84(Suppl. 1):628. (Abstr.)

16. Gruss, H.-J., N. Boiani, D.E. Williams, R.J. Armitage, C.A. Smith, and R.G. Goodwin. 1994. Pleiotropic effects of the CD30 ligand on CD30-expressing cells and lymphoma cell lines. *Blood.* 83:2045–2051.

17. Del Prete, G., M. De Cali, C. Mastromauro, D. Macchia, R. Biagiotti, M. Ricci, and S. Romagnani. 1991. Purified protein derivative of *Mycobacterium tuberculosis* and excretory/secretory antigen(s) of *Toxocara canis* expand in vitro human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. *J. Clin. Invest.* 88:346–350.

18. Schwab, U., H. Stein, J. Gerdes, H. Lemke, H. Kirchner, M. Schaadt, and V. Diehl. 1982. Production of a monoclonal antibody specific for Hodgkin's and Sternberg-Reed cells of Hodgkin's disease and a subset of normal lymphoid cells. *Nature (Lond.).* 299:65–67.

19. Stein, H., D.Y. Mason, J. Gerdes, N. O'Connor, J. Wainscoat, G. Pallesen, K. Gatter, B. Falini, G. Delson, H. Lemke, et al. 1985. The expression of Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that the Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. *Blood.* 66:848–858.

20. Nawrocki, J.P., E.S. Kirsten, and R.I. Fisher. 1988. Biochemical and structural properties of a Hodgkin's disease-related membrane protein. *J. Immunol.* 141:672–680.

21. Josimovic-Alasevic, O., H. Durkop, R. Schwarting, E. Backe, H. Stein, and T. Diamantstein. 1989. Ki-1 (CD30) antigen is released by Ki-1 positive tumor cells in vitro and in vivo. I. Partial characterization of soluble Ki-1 antigen and detection of the antigen in cell culture supernatants and in serum by an enzyme-linked immunosorbent assay. *Eur. J. Immunol.* 19:157–162.

22. Andreesen, R., J. Osterholz, G.W. Lohr, and K.J. Bross. 1984. A Hodgkin cell-specific antigen is expressed on a subset of auto- and alloactivated T (helper) lymphoblasts. *Blood.* 63:1299–1302.

23. Ellis, T.M., P.E. Simms, D.J. Slivnick, H.-M. Jack, and R.I. Fisher. 1993. CD30 is a signal-transducing molecule that defines a subset of human activated CD45RO⁺ T cells. *J. Immunol.* 151:2380–2389.

24. Alzona, M., H.-M. Jack, R.I. Fisher, and T.M. Ellis. 1994. CD30 defines a subset of activated human T cells that produce IFN-γ and IL-5 and exhibit enhanced B cell helper activity. *J. Immunol.* 153:2861–2867.

25. Del Prete, G., E. Maggi, G. Pizzolo, and S. Romagnani. 1995. CD30, Th2 cytokines and HIV infection: a complex and fascinating link. *Immunol. Today.* 16:76–80.

26. Alzona, M., H.M. Jack, R.I. Fisher, and T.M. Ellis. 1995. IL-12 activates IFN-γ production through the preferential activation of CD30⁺ T cells. *J. Immunol.* 154:9–16.

27. Hans, R., G.J. Freeman, Z.B. Wolf, C.D. Gimmi, B. Benacerraf, and L.M. Nadler. 1992. Murine B7 antigen provides an efficient costimulatory signal for activation of murine T lymphocytes via the T cell receptor/CD3 complex. *Proc. Natl. Acad. Sci. USA.* 89:271–275.

28. Tan, P., C. Anasetti, J.A. Hansen, J. Melrose, M. Brunvand, J. Bradshaw, J.A. Ledbetter, and P.S. Linsey. 1993. Induction of alloantigen-specific hyporesponsiveness in human T lymphocytes by blocking interaction of CD28 with its natural ligand B7/BB1. *J. Exp. Med.* 177:165–174.

29. Kurt-Jones, E.A., S. Hamberg, J. Ohara, W.E. Paul, and A.K. Abbas. 1987. Heterogeneity of helper/inducer T lymphocytes. I. Lymphokine production and lymphokine responsiveness. *J. Exp. Med.* 166:1774–1787.

30. Weaver, C.T., C.M. Hawrylowicz, and E.R. Unanue. 1988. T helper cell subsets require the expression of distinct costimulatory signals by antigen-presenting cells. *Proc. Natl. Acad. Sci. USA.* 85:8181–8185.

31. Jenkins, M.K., C.A. Chen, G. Jung, D.L. Mueller, and R.H. Schwartz. 1990. Inhibition of antigen-specific proliferation of type I murine T cell clones after stimulation with immobilized anti-CD3 monoclonal antibody. *J. Immunol.* 144:16–22.

32. Harding, F.A., J.G. McArthur, J.A. Gross, D.H. Raulet, and J.P. Allison. 1992. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature (Lond.).* 356:607–609.

33. Gajewski, T.F., D.W. Lancki, R. Stack, and F.W. Fitch. 1994. "Anergy" of Th0 helper T lymphocytes induces downregulation of Th1 characteristics and a transition to a Th2-like phenotype. *J. Exp. Med.* 179:481–488.

34. Corry, D.B., S.L. Reiner, P.S. Linsley, and R.M. Locksley. 1994. Differential effects of blockade of CD28-B7 on the development of Th1 or Th2 effector cells in experimental Leishmaniasis. *J. Immunol.* 153:4142–4148.