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CD40–CD40 ligand (CD154) engagement is required but may not be sufficient for human T helper 1 cell induction of interleukin-2- or interleukin-15-driven, contact-dependent, interleukin-1β production by monocytes

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SUMMARY

To investigate whether antigen-independent, interleukin-2 (IL-2) or IL-15 activation of polarized T helper (Th) cells would result in contact-dependent activation of monocytes, living Th1 and Th2 cell clones were co-cultured with THP-1 cells or fresh peripheral blood monocytes. Under these conditions IL-1β production was induced almost exclusively by Th1 cells and was dependent on the presence and dose of IL-2 or IL-15, and on cell–cell contact, as demonstrated by double-chamber cultures. Low levels of IL-1 receptor antagonist (IL-1Ra) were induced by Th1 and higher levels by Th2 cells. IL-10 production was similar in Th1/monocyte and Th2/monocyte co-cultures, thus arguing against preferential down-regulation of IL-1β production by anti-inflammatory IL-10 in Th2 co-cultures. In addition, IL-4 and IL-10 neutralization did not result in enhanced IL-1β production in Th2/monocyte co-cultures. Preferential expression on Th1 cells of CD11b correlated with their capacity to induce IL-1β production by THP-1 cells in the presence of IL-2 or IL-15, but anti-CD11b monoclonal antibody could not inhibit this activity. Blockade of the CD40–CD40 ligand interaction resulted in inhibition of IL-1β-inducing capacity while IL-1Ra induction was unaffected, a result previously unknown. This differential effect indicates the selective relevance of CD40–CD40 ligand engagement in inflammatory monocyte responses upon activation by T cells. CD40 ligand expression levels did not differ in Th1 and Th2 cell clones, thus indicating that additional, unidentified molecule(s) preferentially expressed by Th1 cells are involved in their IL-1β induction capacity.

INTRODUCTION

Direct cell–cell contact with activated T cells has been shown to induce monocytes to produce an array of pro-inflammatory or anti-inflammatory molecules (reviewed in ref. 1). Polarized T helper cells (Th) deliver contact-dependent activating stimuli which result in qualitatively different monocyte responses. Antigen-dependent activation of human Th1 but not Th2 cells induces tissue factor production by monocytes.[2] Interleukin-1β (IL-1β) production by monocytes is preferentially induced by Th1 clones while IL-1 receptor antagonist (IL-1Ra) is induced by Th2 clones.[3] As opposed to antigen-dependent activation, unfractionated T cells or synovial T cells from rheumatoid patients have been shown to induce, in an antigen-independent manner, contact-dependent monocyte production of tumour necrosis factor-α (TNF-α) or IL-12 when exposed to IL-15 or to IL-2 in conjunction with TNF-α and IL-6.[4–6] This T-cell activity was inhibited by antibodies directed against CD69, lymphocyte function-associated antigen-1, and intercellular adhesion molecule-1 (ICAM-1).[4] Interestingly, IL-15 and IL-2 share many biological activities on T cells. In contrast to IL-2 however, IL-15 is not produced by T cells but by stromal cells, such as fibroblasts and activated monocytes.[7] IL-15 is present in a membrane-bound, biologically active form on mononuclear phagocytes,[8] and is abundant in areas of chronic inflammation.

The aim of the present study was to investigate whether Th cells with polarized phenotype, when cultured in the presence of IL-2 or IL-15, would induce contact-dependent IL-1β and IL-1Ra production by THP-1 cells or by monocytes in an

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Abbreviations: IL-1Ra, interleukin-1 receptor antagonist; mAb, monoclonal antibody; MFI, mean fluorescence intensity; sCD40-Fc, soluble CD40 linked to an immunoglobulin G1 Fc; TNF-α, tumour necrosis factor-α.

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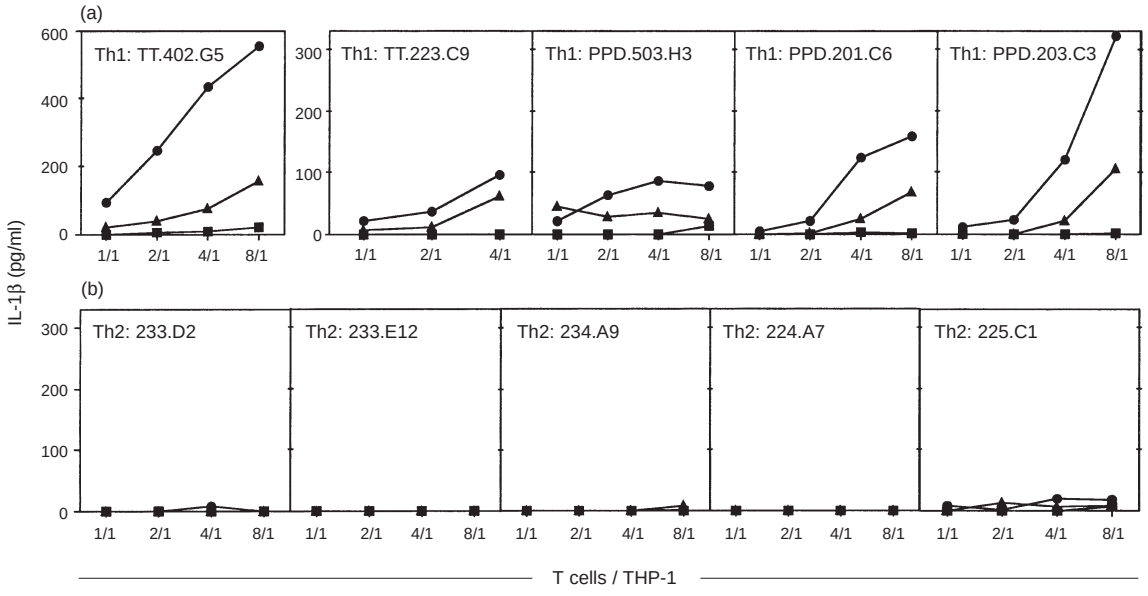

Figure 1. IL-1β production in co-cultures of THP-1 cells with Th1 and Th2 clones in the presence of IL-2 and IL-15; (a) Th1 cell clones, (b) Th2 cell clones. T-cell clones harvested 13 days after previous stimulation were co-cultured (5, 10, 20, or 40×10^4 cells) with THP-1 cells (5×10^4 cells) in medium alone (■), in the presence of IL-2 (20 U/ml) (▲), or IL-15 (100 ng/ml) (●). After 48 hr of culture, supernatants were harvested and analysed for IL-1β content.

antigen-independent manner. Based on the observation that living Th1, but not Th2, cells induced IL-1β production by monocytes, we tested whether cytokines or surface molecules differentially expressed in polarized Th cells could explain their different behaviour in this model of inflammation.

MATERIALS AND METHODS

Media, reagents and antibodies

Penicillin, streptomycin, RPMI-1640, fetal calf serum (FCS) and phosphate-buffered saline (PBS) were from Gibco (Paisley, UK). Human AB serum was provided by the Blood Bank of the University Hospital (Geneva, Switzerland). Recombinant human IL-2 and interferon-γ (IFN-γ) were from Biogen (Cambridge, MA); IL-15 and sCD40-Fc from Immunex (Seattle, WA). Ficoll–Paque was from Pharmacia (Uppsala, Sweden); phytohaemagglutinin (PHA) from EY Laboratories (San Mateo, CA); protein-purified derivative (PPD) from Statens Serum Institut (Copenhagen, Denmark); tetanus toxoid (TT) from Behringwerke (Marburg, Germany); sheep red blood cells from Biomérieux (Lyon, France); neuraminidase, polymyxin B and nonidet-P40 (NP-40) from Sigma (St Louis, MO); and $1,25(OH)_2$ vitamin D_3 from Hoffmann-La Roche (Basel, Switzerland). The OKT3 monoclonal antibody (mAb) was a gift from Cilag (Herenthals, Belgium). Anti-CD3, -CD4, -CD8, -CD11b (immunoglobulin G1; IgG1), -CD14, -CD18, -CD19, -CD16, -CD25, -CD18 mAb, irrelevant, isotype-matched controls, and fluorescein isothiocyanate (FITC) –anti-mouse immunoglobulin were from Dako (Copenhagen, Denmark); anti-CD11a, -CD11b (IgG1), -CD11c mAb from Immunotech (Marseille, France); anti-CD69 mAb from Becton Dickinson (San Jose, CA), anti-CD11b (IgM) from Coulter (Miami, FL); anti-IL-2Rβ (CF1) and anti-IL-2Rγ (3B5) mAb from Dr J. Thèze (Institut Pasteur, Paris, France); anti-CD40 ligand (CD40L; CD154) clone 5C8 was from Dr P. E. Lipsky (University of Texas, Dallas, TX), anti-IFN-γ was from Dr G. Garotta (Human Genome Sciences, Rockville, MD), anti-IL-4 and anti-IL-10 mAb from DNAX (Palo Alto, CA).

T-cell clones

T-cell clones were generated upon antigen-priming (PPD or TT), cloning and expansion in the presence of IL-2 as described.[3] Seven to 15 days after previous stimulation, T-cell clones were washed extensively before use. For cytokine production 5×10^4 T cells were cultured in 96-well flat-bottom plates in medium alone or with plate-bound OKT3 mAb (coated overnight at 4° at 0·5 μg/ml). IL-1β, IFN-γ, and IL-4 were determined in 24-hr culture supernatants. High IFN-γ/low IL-4 producers were defined Th1, whereas Th2 were low IFN-γ/high IL-4 producers.[3]

Monocytes

The human monocytic cell line THP-1 (American Type Culture Collection, Manassas, VA) was maintained in RPMI-1640 supplemented with 10% FCS. Prior to cell-contact experiments, cells were cultured for 48 hr in the presence of 4 ng/ml of $1,25(OH)_2$ vitamin D_3 to enhance their response to activation stimuli.[9] Fresh monocytes were obtained by aggregation in the cold in the presence of polymyxin B (1 μg/ml) as described.[10] Monocyte-enriched aggregates were further depleted of T and natural killer (NK) cells by rosetting with neuraminidase-treated sheep red blood cells. Monocyte purity routinely consisted of $>85\%$ $CD14^+$ cells, $<1{\cdot}5\%$ $CD3^+$ cells, $<1\%$ $CD19^+$ cells.

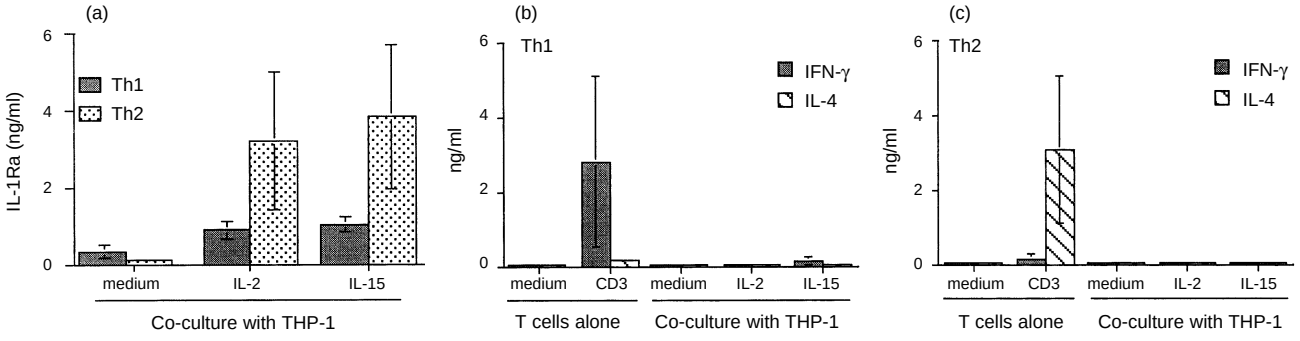

Figure 2. IL-1Ra, IFN-γ and IL-4 production in T/THP-1 cell co-cultures and IFN-γ/IL-4 production by Th1 and Th2 clones. (a) IL-1Ra produced in co-cultures of Th1 or Th2 with THP-1 cells; (b) IFN-γ and IL-4 production by Th1 clones; (c) IFN-γ and IL-4 production by Th2 clones. Co-culture conditions were the same as reported in Figure 1, and IL-1Ra, IFN-γ and IL-4 were measured in 48 hr supernatants at the highest T : THP-1 ratio. T-cell clones were also cultured alone (5×10^4 cells/well) and activated by CD3-cross-linking. IFN-γ and IL-4 were measured in 24 hr supernatants. Data are means ± SEM of five Th1 and five Th2.

Co-cultures of T cells with monocytes

THP-1 cells were seeded at 5×10^4 cells/well in 96-well flat-bottom plates. Fresh monocytes were seeded at the same concentration in medium containing 1 μg/ml polymyxin B. T-cell clones were co-cultured with either target cell at T-cell : monocyte ratios ranging from 1 : 1 to 8 : 1 in 200-μl final volume of RPMI-1640 with 10% FCS. After 48 hr of culture, supernatants were harvested and stored at −20° for further cytokine determination. In some assays anti-CD11b mAb (Dako, IgG1; Immunotech IgG1; Coulter, IgM), anti-CD40L, anti-IFN-γ, anti-IL-4, anti-IL-10, or irrelevant immunoglobulin were added at 10 μg/ml to the co-cultures with IL-2 or IL-15. The construct sCD40-Fc was used at 1 μg/ml, and IFN-γ at 300 U/ml. In selected experiments, T cells and monocytes were preincubated separately with CD11b mAb and washed before being co-cultured. In double-chamber experiments, 6·5-mm Transwell units (Costar, MA) equipped with semipermeable membranes were used in 24-well plates. T cells were seeded in the lower chamber (2·5, 5 and 10×10^5 cells/well) and THP-1 cells ($2{\cdot}5 \times 10^5$ cells/well) were seeded either in the upper or lower chamber in a 1-ml final volume. In all cases, THP-1 cells and T cells alone were used as controls.

Flow cytometric analysis

T cells were incubated with appropriate dilutions of unconjugated mouse mAbs and were further incubated with FITC-conjugated goat anti-mouse immunoglobulin. The mean fluorescence intensity (MFI) was recorded upon gating of living cells and expressed in arbitrary units on a four-decade logarithmic scale.

Cytokine determination

Production of IL-1β (Immunotech, Marseille, France), IL-1Ra, IL-10 (R & D Systems, Minneapolis, MN), IFN-γ and IL-4 (Hoffmann La-Roche, Basel, Switzerland) was assessed by enzyme-linked immunosorbent assay (ELISA).[11] The sensitivity threshold was 2·5 pg/ml for IL-1β, 8 pg/ml for IL-10 and 25 pg/ml for IL-1Ra, IFN-γ and IL-4.

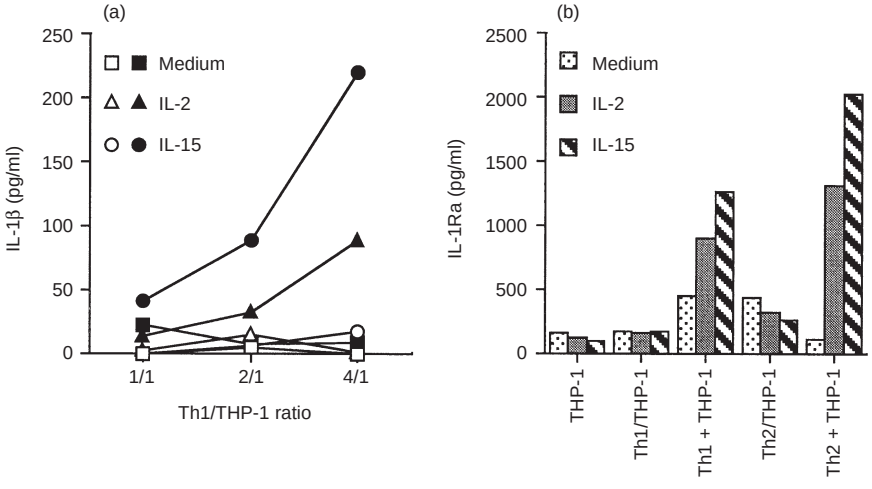

Figure 3. Cell–cell contact is required for IL-1β and IL-1Ra production in Th1/THP-1 co-cultures. T cells (2·5, 5, or 10×10^5 cells) and THP-1 cells ($2{\cdot}5 \times 10^5$ cells) were co-cultured in a double-chamber 24-well culture plate either together (filled symbols) or separated by a semi-permeable membrane (open symbols). IL-1β and IL-1Ra production was measured in the supernatants after 48 hr of culture. IL-15 (100 ng/ml), IL-2 (20 IU/ml), medium. Similar data were obtained in two distinct experiments.

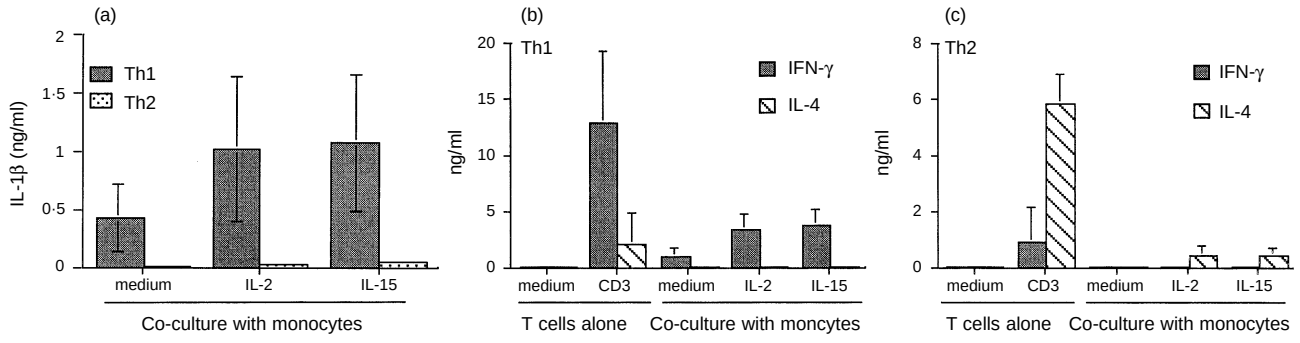

Figure 4. IL-1β, IFN-γ and IL-4 production in T/monocyte co-cultures and IFN-γ/IL-4 production by Th1 and Th2 clones. (a) IL-1β produced in co-cultures of Th1 or Th2 with monocytes; (b) IFN-γ and IL-4 production by Th1 clones; (c) IFN-γ and IL-4 production by Th2 clones. Culture conditions were specified in legends of Figs 1 and 2 except that freshly isolated peripheral blood monocytes were used instead of THP-1 cells. Data are means ± SEM obtained with three Th1 and three Th2 clones.

Statistical analysis

The non-parametric Mann–Whitney *U*-test was used to compare means, and the Spearman rank test to study correlations.

RESULTS

Co-culture of THP-1 cells with Th1 but not with Th2 cells in the presence of IL-2/IL-15 results in IL-1β production

Low or undetectable levels of IL-1β were released in co-cultures of THP-1 cells with Th cells in the absence of exogenous cytokines (Fig. 1). IL-1β was produced when THP-1 cells were co-cultured with Th1 clones in the presence of IL-2 or IL-15. IL-1β production increased in a dose-dependent manner with increasing numbers of T cells (Fig. 1) and increasing concentrations of IL-2 or IL-15, reaching a plateau at 20 U/ml of IL-2 and 10–100 ng/ml of IL-15. In contrast, Th2 clones, whether stimulated or not by IL-2 or IL-15, failed to induce, or were poor inducers of, IL-1β production (Fig. 1). No IL-1β was detected when THP-1 or T cells were cultured alone with or without IL-2 or IL-15, nor in supernatants of T cells activated by CD3 cross-linking. In contrast, IL-1Ra was detected in THP-1 co-cultures with both Th1 and Th2 cells in the presence of IL-2 or IL-15 (Fig. 2a). IL-1Ra levels were higher in co-cultures with Th2 than in those with Th1 cells, but the difference was not statistically significant in these experiments. Interestingly, although THP-1 cells and T-cell clones were fully allogeneic, very little IFN-γ or IL-4, if any, was produced both in the presence and absence of IL-2 or IL-15 (Fig. 2b,c). In parallel experiments high levels of IFN-γ or IL-4 were induced when T-cell clones were activated by CD3-cross-linking (Fig. 2b,c). Experiments were performed in which Th cells were cultured in contact with THP-1 cells or separated by a semi-permeable membrane. IL-1β production was observed only when Th1/THP-1 cells were cultured in physical contact and in the presence of IL-2 or IL-15 (Fig. 3a). IL-1Ra induction also required contact and was observed with both Th1 and Th2 cells (Fig. 3b).

The high-affinity IL-2 receptor (CD25) is transiently expressed upon antigen-recognition by T cells. At any given time after re-stimulation, Th2 clones expressed higher levels of CD25 per cell than did Th1 (Table 1). The surface expression of the β-chains (CD122) and γ-chains (CD132) of the IL-2R shared by IL-2 and IL-15 was also tested and found to be similar in Th1 and Th2 clones (Table 1).

Contact with peripheral blood monocytes

In additional experiments freshly separated, allogeneic or syngeneic, peripheral blood monocytes were used instead of THP-1 cells. Again, IL-1β production was observed when monocytes were co-cultured with Th1 but not with Th2 cells (Fig. 4a). IL-1Ra levels were higher in co-cultures with Th2 than with Th1 cells. In the presence of IL-2 (20 U/ml) IL-1Ra was 15·8 ± SD 3·1 ng/ml in Th1/monocyte co-cultures and 26·4 ± 3·2 ng/ml in Th2/monocyte co-cultures ($P < 0{\cdot}02$). At variance with the experiments involving THP-1 cells, Th/monocyte co-cultures resulted in IFN-γ or IL-4 production and with seven Th1 clones out of 16 tested no exogenous IL-2 or IL-15 was required to induce IL-1β production. With all the clones the levels of IL-1β, IFN-γ and IL-4 were increased when exogenous IL-2 or IL-15 was added (Fig. 4a–c).

IL-10 production in Th/monocyte co-cultures

When measured in co-culture supernatants IL-10 levels were undetectable in Th1/THP-1 as well as in Th2/THP-1 co-culture with or without IL-2 or IL-15 (not shown). The IL-10 levels measured in co-cultures of Th cells with freshly isolated peripheral monocytes varied according to the monocyte donor,

Table 1. Levels of various cell surface markers on Th1 and Th2 clones

Marker	Th1 MFI	*n**	Th2 MFI	*n*
Control	0·18	18	0·18	11
CD11a	22·1	8	22·2	6
CD11b	0·52†	18	0·28	11
CD11c	0·44	8	0·36	5
CD18	26·3	8	19·1	5
CD25	1·52†	16	4·74	11
CD69	3·08†	18	5·34	11
CD122	0·70	2	0·69	2
CD132	3·12	2	3·19	2

*Numbers of clones tested; †$P < 0{\cdot}01$ compared to Th2 as assessed by the Mann–Whitney *U*-test.

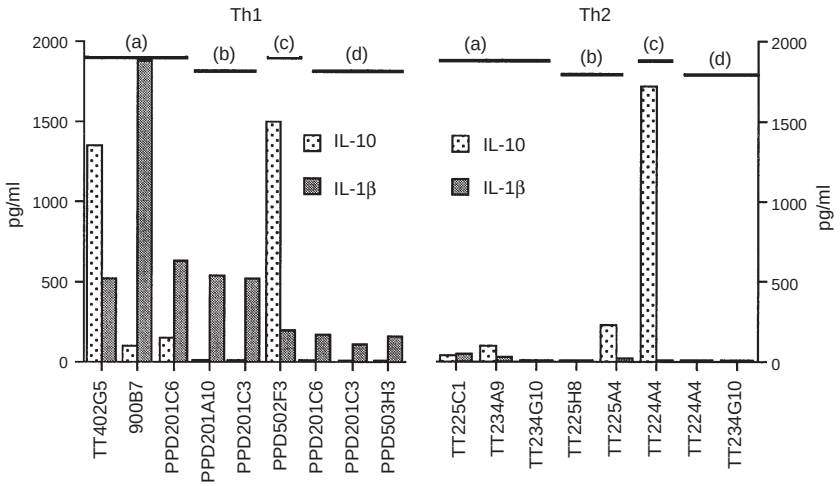

Figure 5. IL-1β and IL-10 production in T/monocyte co-cultures. Freshly isolated monocytes (5×10^4 cells/well) were cultured with T cells (4×10^5 cells/well) in the presence of IL-2 (20 U/ml) for 48 hr. Data are results from single clones obtained in four distinct experiments (a–d) where Th1 and Th2 cell clones were cultured in parallel. Letters in brackets indicate distinct monocyte donors. In experiments (b) and (c) monocytes and T cells were autologous.

but again no significant differences were observed when comparing Th2/monocyte to Th1/monocyte co-cultures. In fact, IL-10 levels in the presence of IL-2 (20 U/ml) were undetectable in four of eight Th2/monocyte co-cultures and in five of nine Th1/monocyte co-cultures (Fig. 5).

Additional experiments were conducted in which neutralizing anti-IL-10 or anti-IL-4 mAb alone or together were added to Th2/monocyte co-cultures. In no case did IL-10 and IL-4 neutralization result in enhanced IL-1β production (three Th2 clones tested).

Th1 clones express higher levels of CD11b and lower levels of CD69 than Th2 clones

Both β_2-integrins and CD69 have previously been shown to be involved in contact-dependent activation of monocytes.[5,9,12,13] CD11b (α_M) expression was negative or low on Th2 cells while it was high on Th1 cells ($P < 0{\cdot}01$) (Table 1). The β-chain CD18, which CD11b uses to form $\alpha_M\beta_2$-integrin, was equally expressed on both Th1 and Th2 cell clones as was α_L CD11a. CD11c (α_X) levels tended to be higher on Th1 than on Th2 cell clones, a difference that was not statistically significant. CD69 was expressed on both Th1 and Th2 cells, but at higher levels on Th2 cell clones. The differential expression of CD11b on polarized T cells strongly correlated with IL-1β production in T/THP-1 co-cultures in the presence of IL-2 or IL-15 ($P = 0{\cdot}008$ in IL-2, $P = 0{\cdot}004$ in IL-15). However, when three different anti-CD11b mAb were tested in the Th1/monocyte co-culture no significant inhibition was observed with five distinct clones, regardless of whether the mAb were added during co-culture or whether Th1 or monocytes were preincubated with the mAb before the co-culture (Fig. 6a).

CD40–CD40L interaction is involved in Th1/monocyte contact

CD40L expression was similar in Th1 and Th2 clones and although it decreased with time it was still detectable 10–15 days after subculture when the clones were assayed in

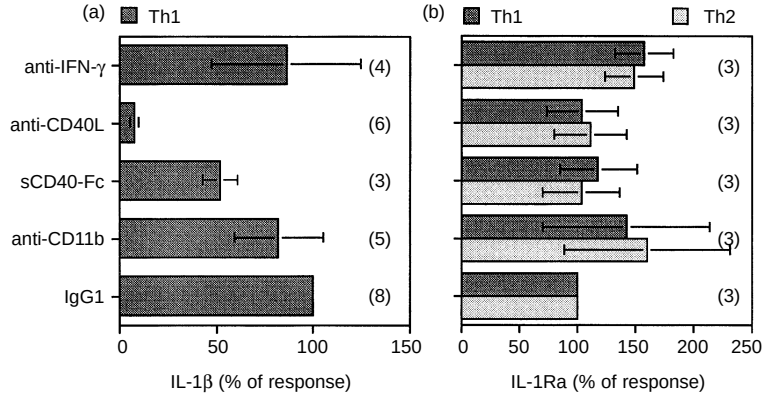

Figure 6. Effect of various mAb on IL-1β production in Th1/monocyte co-cultures. (a) IL-1β production in Th/monocyte co-cultures. (b) IL-1Ra production in Th1/monocyte or Th2/monocyte co-cultures. Freshly isolated monocytes were cultured with Th1 cells as described in Figure 5. The mAb or sCD40-Fc were added at the beginning of the culture. All the mAb were IgG1 and used at 10 μg/ml. The sCD40 was used at 1 μg/ml. The number of individual clones tested is given in parentheses. IL-1β production in the presence of irrelevant IgG1 was $153{\cdot}5 \pm 35{\cdot}4$ in different experiments. Bars indicate SEM.

co-cultures with monocytes (Fig. 7). When the 5C8 anti-CD40L mAb was added to the Th1/monocyte co-cultures it decreased by more than 80% of IL-1β production (Fig. 6a). Moreover, sCD40-Fc in Th1/monocyte co-cultures inhibited IL-1β production by approximately 50% (Fig. 6a). IL-1Ra production under the same culture conditions was unaffected by CD40–CD40L blockade, both with Th1 and Th2 cells (Fig. 6b). In the same Th1/monocyte co-cultures where anti-CD40L mAb decreased IL-1β production, the levels of IFN-γ were variably affected (unchanged with three Th1 clones and decreased with three other Th1 clones), nor did neutralization of IFN-γ decrease IL-1β production (Fig. 6a). The addition of exogenous IFN-γ to Th2/monocyte co-cultures did not enhance IL-1β production (four of four Th2/monocyte co-cultures).

DISCUSSION

The present study shows that T-cell clones belonging to the Th1 subset and expressing CD11b induce cell-contact-dependent IL-1β production by monocytes in an antigen-independent manner. Our results are in agreement with, and extend, recent work showing that T cells activated in the presence of IL-2 or IL-15 induce TNF-α and IL-12 production by monocytes.[4–6] More important, heterogeneity in Th subsets resulted in dramatic, not yet described, differences in their capacity to induce IL-1β. Indeed, Th1 cells were much more potent inducers than Th2 cells.

CD40–CD40L interaction is of relevance in several physiopathological events involving cognate interactions between T cells and mononuclear phagocytes.[6,14–16] CD40L has been shown to be transiently expressed on the $CD4^+$ T-cell surface upon antigen activation, peaking at 6 hr and vanishing thereafter.[17] In our culture conditions its expression was retained for at least 10 days, and was not substantially different in Th1 and Th2 clones. Since similar surface expression of CD40L on Th1 and Th2 clones was not paralleled by equivalent biological activity in contact-dependent activation of monocytes CD40–CD40L engagement is required but is not *per se* sufficient to induce IL-1β production by monocytes in an IL-2/IL-15-driven system.

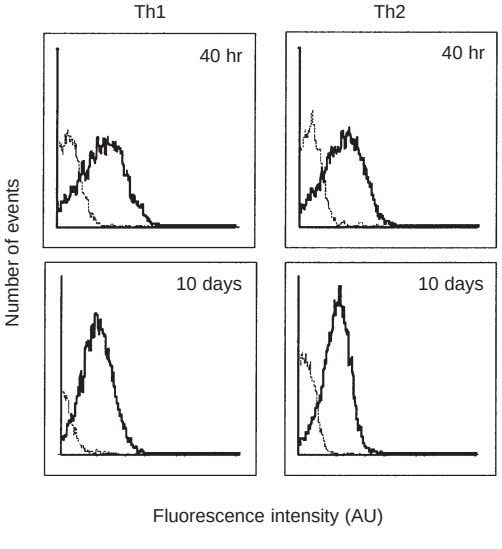

Figure 7. Representative CD40L expression on Th1 and Th2 clones as function of time. Flow cytometry was performed on Th1 and Th2 cells simultaneously 40 hr (upper panel) or 10 days (lower panel) after subculture. Thick line: CD40L, dotted line: isotype control.

Several not mutually exclusive mechanisms may account for the differences observed using Th1 and Th2 cell clones. An explanation could be related to the preferential production of IFN-γ by Th1 cells. A large body of evidence indicates that this may not necessarily be the case. First, in Th1/THP-1 co-cultures where IL-1β production was substantial in the presence of IL-2 or IL-15, IFN-γ levels were undetectable or low. Second, IFN-γ neutralization in Th1/monocyte co-cultures where the levels of IFN-γ were high did not consistently reduce IL-1β production. Indeed, clonal heterogeneity was observed in this respect. Finally, in Th2/monocyte co-culture the addition of exogenous IFN-γ did not result in enhanced IL-1β production (not shown). A second mechanism could be related to the preferential production by Th2 cells of IL-4 or IL-10, cytokines with anti-inflammatory properties and capable of inhibiting IL-1β production.[18,19] This possibility appears unsubstantiated. In fact, IL-4 and IL-10 production were undetectable or low in Th2/THP-1 as well as in Th1/THP-1 co-cultures and neutralizing antibody to IL-4 and IL-10 did not result in enhanced IL-1β production in Th2/monocyte co-cultures. In addition, while IL-10 was detected in some but not all Th2/monocyte co-cultures, the levels of IL-10 detected in Th1/monocyte co-cultures were similar. Noticeably, in an experimental system similar to ours, Poe *et al.* have shown that when IL-4 and/or IL-10 are added simultaneously with activated T cells on monocytes only a slight reduction in IL-1β mRNA is observed.[17] A third possible mechanism could be related to the preferential expression on Th1 cells of additional surface molecule(s) or their preferential production of soluble factors which could act as inducer(s) of monocyte activation in conjunction with CD40L. Along this line it is worthwhile to stress that Th1 cell clones preferentially expressed the β_2-integrin CD11b, previously described in a subset of CD45RO $CD4^+$ T cells,[20] and that a strong statistical correlation was observed between CD11b expression and IL-1β production. We were however, unable to inhibit Th1/monocyte co-culture by using three different anti-CD11b mAb. The CD11b/CD18 integrin has been shown to bind to several different molecules such as C3bi, ICAM-1, glucan and CD23.[21] In addition, CD11b on neutrophils and monocytes plays a role as co-receptor of several glycosyl-phosphatydil-inositol (GPI)-anchored receptors including CD14, uPAR (CD87), FcγRIII (CD16b) and of the FcγRIIa (CD32).[22] Thus, the preferential expression of CD11b on Th1 cells could result in the enhanced binding to one or more of its numerous ligands, but it could also result in the preferential involvement of other receptors through its *cis*-acting function. In depth investigation is therefore needed to characterize better the role of CD11b on Th1 cells.

IL-2 and IL-15 have been shown to enhance human and murine monocyte/macrophage activities.[23,24] In particular, IL-15 has been shown to function as an autocrine regulator of lipopolysaccharide-activated murine macrophage pro-inflammatory cytokine production.[24] The co-culture assay of living cells adopted in this study did not reveal whether the monocyte response was due to soluble factors in association with cell–cell contact, or whether cell–cell contact alone was

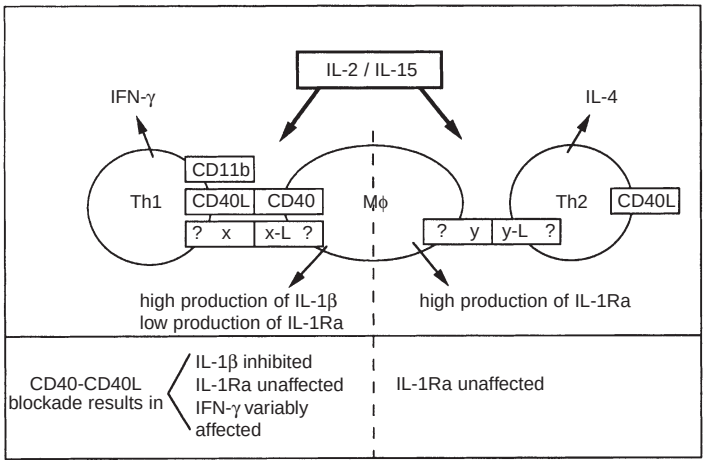

Figure 8. Schematic representation of Th1/monocyte and Th2/monocyte co-culture outcomes. x, x-L, y, y-L denote hypothetical receptors and receptor ligands.

sufficient for monocyte responses. However, when T cells and THP-1 cells were separated by a semi-permeable membrane no IL-1β was detected and IL-1Ra production was not enhanced, which is in agreement with previous data demonstrating that cell–cell contact is required for both cytokines.[4,5,9] However, while IL-1β induction was inhibited by CD40–CD40L blockade IL-1Ra was not. This is consistent with data showing that intracellular pathways involved in IL-1Ra induction differ from those involved in IL-1β induction and indicate that various surface molecules may be at play in cell–cell interactions.[3,25]

Th2 clones were found to express higher levels of CD69 and CD25 than Th1 clones. Since Th1 and Th2 clones were cultured under identical conditions and tested at similar times after re-stimulation it appears that the higher expression of CD25 and CD69 is an intrinsic characteristic of Th2 clones. CD69 gene expression is under the control of several transcription factors, among them members of the GATA family.[26] It has recently been shown that GATA-3 is necessary and sufficient for Th2 cytokine gene expression on CD4 T cells.[27] Thus, it is possible that CD4 T-cell differentiation toward Th2 phenotype is accompanied by higher levels of CD69 expression since GATA-3 is selectively retained in Th2 cells. Several reports have indicated that CD69 is involved in the contact-dependent activation of monocytes by T cells based on the inhibitory activity of anti-CD69 mAb.[5,12,13] The higher levels of CD69 found on Th2 cells, poor inducers of IL-1β production in our experimental model, suggest that CD69 on T cells may be a molecule that is necessary while not sufficient for activatory signalling.

In conclusion, the data gathered in the present study support the contention that IL-2 and IL-15 may generate an amplification loop in T helper/mononuclear phagocyte cross-talk. Pro-inflammatory IL-1β production is selectively induced by Th1 cells and is dependent on CD40–CD40L engagement, while IL-1Ra is preferentially induced by Th2 cells independently of CD40–CD40L interaction (Fig. 8). This contact-dependent mechanism may operate in an antigen-independent manner, modulating chronic inflammation.

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REFERENCES

1. Burger D. & Dayer J.M. (1998) Interactions between T cell plasma membranes and monocytes. In: *T Cells in Arthritis* (ed. P. Miossec, W. B. Van den Berg & G. S. Firestein), p. 111. Birkhäuser Verlag, Basel.
2. Del Prete G., De Carli M., Lammel R.M. *et al.* (1995) Th1 and Th2 T-helper cells exert opposite regulatory effects on procoagulant activity and tissue factor production by human monocytes. *Blood* **86,** 250.
3. Chizzolini C., Chicheportiche R., Burger D. & Dayer J.-M. (1997) Human Th1 cells preferentially induce interleukin (IL) -1β while Th2 cells induce IL-1 receptor antagonist production upon cell/cell contact with monocytes. *Eur J Immunol* **27,** 171.
4. Sebbag M., Parry S.L., Brennan F.M. & Feldmann M. (1997) Cytokine stimulation of T lymphocytes regulates their capacity to induce monocyte production of tumor necrosis factor-alpha, but not interleukin-10: possible relevance to pathophysiology of rheumatoid arthritis. *Eur J Immunol* **27,** 624.
5. McInnes I.B., Leung B.P., Sturrock R.D., Field M. & Liew F.Y. (1997) Interleukin-15 mediates T cell-dependent regulation of tumor necrosis factor-alpha production in rheumatoid arthritis. *Nat Med* **3,** 189.
6. Avice M.N., Demeure C.E., Delespesse G., Rubio M., Armant M. & Sarfati M. (1998) IL-15 promotes IL-12 production by human monocytes via T cell-dependent contact and may contribute to IL-12-mediated IFN-gamma secretion by CD4+ T cells in the absence of TCR ligation. *J Immunol* **161,** 3408.
7. Waldmann T., Tagaya Y. & Bamford R. (1998) Interleukin-2, interleukin-15, and their receptors. *Int Rev Immunol* **16,** 205.

8. Musso T., Calosso L., Zucca M. *et al.* (1999) Human monocytes constitutively express membrane-bound, biologically active, and interferon-gamma-upregulated interleukin-15. *Blood* **93,** 3531.
9. Vey E., Zhang J.H. & Dayer J.M. (1992) IFN-gamma and 1,25(OH)2D3 induce on THP-1 cells distinct patterns of cell surface antigen expression, cytokine production, and responsiveness to contact with activated T cells. *J Immunol* **149,** 2040.
10. Armant M., Rubio M., Delespesse G. & Sarfati M. (1995) Soluble CD23 directly activates monocytes to contribute to the antigen-independent stimulation of resting T cells. *J Immunol* **155,** 4868.
11. Gallati H., Pracht I., Schmidt J., Haring P. & Garotta G. (1987) A simple, rapid and large capacity ELISA for biologically active native and recombinant human IFN gamma. *J Biol Regul Homeost Agents* **1,** 109.
12. Isler P., Vey E., Zhang J.H. & Dayer J.M. (1993) Cell surface glycoproteins expressed on activated human T cells induce production of interleukin-1 beta by monocytic cells: a possible role of CD69. *Eur Cytokine Netw* **4,** 15.
13. Manie S., Kubar J., Limouse M. *et al.* (1993) CD3-stimulated Jurkat T cells mediate IL-1 beta production in monocytic THP-1 cells. Role of LFA-1 molecule and participation of CD69 T cell antigen. *Eur Cytokine Netw* **4,** 7.
14. Grewal I.S. & Flavel R.A. (1998) CD40 and CD154 in cell-mediated immunity. *Annu Rev Immunol* **16,** 111.
15. Stout R.D., Suttles J., Xu J., Grewal I.S. & Flavell R.A. (1996) Impaired T cell-mediated macrophage activation in CD40 ligand-deficient mice. *J Immunol* **156,** 8.
16. Wagner D.H.J.R., Stout R.D. & Suttles J. (1994) Role of the CD40–CD40 ligand interaction in CD4+ T cell contact-dependent activation of monocyte interleukin-1 synthesis. *Eur J Immunol* **24,** 3148.
17. Poe J.C., Wagner D.H.J.R., Miller R.W., Stout R.D. & Suttles J. (1997) IL-4 and IL-10 modulation of CD40-mediated signaling of monocyte IL-1beta synthesis and rescue from apoptosis. *J Immunol* **159,** 846.
18. Hart P.H., Vitti G.F., Burgess D.R., Whitty G.A., Piccoli D.S. & Hamilton J.A. (1989) Potential antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor alpha, interleukin 1, and prostaglandin E2. *Proc Natl Acad Sci USA* **86,** 3803.
19. De Waal Malefyt R., Abrams J., Bennett B., Figdor C.G. & De Vries J.E. (1991) Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* **174,** 1209.
20. Hamann D., Baars P.A., Hooibrink B. & Van Lier R.W. (1996) Heterogeneity of the human CD4+ T-cell population: two distinct CD4+ T-cell subsets characterized by coexpression of CD45RA and CD45RO isoforms. *Blood* **88,** 3513.
21. Diamond M.S. & Springer T.A. (1994) The dynamic regulation of integrin adhesiveness. *Curr Biol* **4,** 506.
22. Todd R.F. & Petty H.R. (1997) Beta 2 (CD11/CD18) integrins can serve as signaling partners for other leukocyte receptors. *J Lab Clin Med* **129,** 492.
23. Espinoza-Delgado I., Bosco M.C., Musso T., Gusella G.L., Longo D.L. & Varesio L. (1995) Interleukin-2 and human monocyte activation. *J Leukoc Biol* **57,** 13.
24. Alleva D.G., Kaser S.B., Monroy M.A., Fenton M.J. & Beller D.I. (1998) IL-15 functions as a potent autocrine regulator of macrophage proinflammatory cytokine production. Evidence for differential receptor subunit utilization associated with stimulation or inhibition. *J Immunol* **159,** 2941.
25. Vey E., Dayer J.M. & Burger D. (1997) Direct contact with stimulated T cells induces the expression of IL-1beta and IL-1 receptor antagonist in human monocytes. Involvement of serine/-threonine phosphatases in differential regulation. *Cytokine* **9,** 480.
26. Ziegler S.F., Levin S.D., Johnson L. *et al.* (1994) The mouse CD69 gene. Structure, expression, and mapping to the NK gene complex. *J Immunol* **152,** 1228.
27. Zheng W. & Flavell R.A. (1997) The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* **89,** 587.