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Two distinct pathways of human macrophage differentiation are mediated by interferon-γ and interleukin-10

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SUMMARY

Forming cellular conjugates with T cells, macrophages can help their targets to mount an immune response or they can destroy the targeted T cell. The two functions are performed by two distinct macrophage subsets that can be distinguished by cell surface marker phenotypes, $B7^+$ $CD16^-$ and $B7^-$ $CD16^+$. Interferon-γ (IFN-γ) induces the former, interleukin-10 (IL-10) induces the latter phenotype. The two macrophage differentiation pathways are mutually exclusive; each cytokine inhibits the effect of the other cytokine. The second messenger cAMP enhances the macrophage B7 expression and suppresses the macrophage CD16 expression. However, together with IL-10, cAMP blocks the generation of both macrophage phenotypes. In the chimpanzee we noted deviations from this differentiation pattern that are suggestive of an enhanced IL-10 presence in the primate environment.

INTRODUCTION

Macrophages interact with T cells through physical interaction. Forming cellular conjugates with T cells, macrophages present antigen and provide costimulatory, B7-mediated, signals. This enables T cells to mount immune responses. Through the expression of FcRs, macrophages can also engage antibody-reactive T cells in cellular conjugate formation and destroy them.[1–4] The ability of macrophages to perform these functions is controlled by cytokines. Interferon-γ (IFN-γ) enhances the T cell-activating macrophage function by upregulating the expression of major histocompatibility complex (MHC) class II and B7 molecules.[5] Interleukin-10 (IL-10) inhibits the same function.[6–8] The suppression of the $B7^+$ phenotype correlates with the suppression of macrophage accessory cell function.[8]

In a recent analysis of macrophage phenotypes and function in HIV-1-infected individuals it was found that macrophages lose the ability to upregulate the expression of MHC class II and B7 molecules and the ability to stimulate the proliferation of T cells. However, their capacity to destroy antibody-reactive T cells increases at the same time.[3] In particular, macrophages acquire the ability to destroy T cells that bind immune-complexed CD4-reactive HIV-1 envelope molecules. The destruction is inhibitable with CD16-specific mAb, suggesting that it is facilitated, in large part at least, by the FcγIIIR.[4]

The notion that one macrophage function, T-cell stimulation, may decrease while another function, T-cell deletion, increases suggests the existance of distinct macrophage subsets. The experiments described herein were performed to examine the possibility of distinct macrophage differentiation pathways. The results show that IFN-γ induces the differentiation of an immunostimulatory macrophage phenotype and inhibits macrophage differentiation into an immune destructive phenotype. In contrast, IL-10 induces a cytotoxic macrophage phenotype. It enhances CD16 expression and inhibits the B7 expression of macrophage.

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MATERIALS AND METHODS

Cells

Human peripheral blood mononuclear lymphoid cells (PBLC) were isolated by density gradient separation from apparently healthy human or chimpanzee donors. Chimpanzee blood was kindly provided by Dr Preston Marx, The Aron Diamond AIDS Research Primate Center, Tuxedo, NY.

Cell culture

PBLC were placed in 96-well flat-bottom tissue culture plates at a concentration of 2×10^5 cells per 0·1 ml RPMI-1640 medium containing 1% heat-inactivated human serum. The cells were incubated in a humidified incubator at 37° for the length of time indicated in the data displays. In order to recover plastic adherent cells the plates were subjected to a forceful stream of ice-cold balanced salt solution until at least 95% of the attached cells became dislodged.

Reagents

IL-10 was the gift of M. Howard, DNAX Research Institute, Palo Alto, CA. IFN-γ was a gift from S. Ferrone, New York Medical College, Valhalla NY. HIV-1 envelope protein gp120

was given by K. Steimer, Chiron Corp., Emoryville, CA. A monoclonal gp120-specific human antibody was provided by S. Koenig, Medimmune, VA. Anti-CD3 mAb was provided by R. Mittler, Bristol Myer Squibb, Seattle, WA. Dibuturyl cAMP was purchased from Sigma, St Louis, MO.

Flow cytometry

Two-colour fluorescence analysis of macrophages, gated by light scatter,[3] was carried out in the FACScan flow cytometer (Beckton Dickinson, Mountain View, CA) using B7-reactive fluorescein isothiocyanate (FITC)-labelled CTLA-4Ig (generously provided by P. Lindsley, Bristol Myer Scuibb, Seattle, WA) and biotinylated anti-CD16 mAb (clone B73.1), provided by G. Trinchieri, Wistar Institute, Philadelphia, PA). The biotin label was developed by phycoerythrin–streptavidin (Sigma) as described.[9] Heat-inactivated human serum (10%) was added to reduce macrophage background fluorescence. The mean fluorescent units were recorded and are displayed.

Macrophage cytotoxicity assay

Macrophages destroy antibody- or immune complex-targeted T cells through apoptosis.[2–4] A quantitative assay has been developed.[3,4] PBLC (2×10^5/0·1 ml) were placed in 96-well flat-bottom tissue culture plates. When separation of macrophages and lymphocytes was desired, non-adherent (lymphocyte enriched) cells were removed after 60 min and returned to the same or to a different population of plastic adherent (monocyte enriched) cells as indicated. The cultures were incubated for 2 days in the presence or in the absence of gp120 and/or anti-gp120 monoclonal antibody (mAb) as indicated. Harvested cells were immediately counted in an Ultraplane Neubauer Haemacytometer, washed, and double stained with FITC-labelled anti-CD4 mAb (OKT4a) and biotinylated (Streptavidin developed) anti-CD8 mAb (OKT8). The percentage of label-positive living (propidium iodine excluding) cells was determined in the FACScan flow cytometer and the absolute counts of $CD4^+$, $CD8^+$ and double-negative cells was calculated (and is displayed).

RESULTS

Antagonism between IFN-γ and IL-10 in the phenotypic differentiation of macrophages

Human PBLC were cultured alone or in the presence of cytokines, IFN-γ and IL-10, as indicated in Fig. 1. The cells were harvested 1 or 2 days later and stained for analysis in the flow cytometer. The expression of CTLA-4Ig-reactive B7 molecules[10] and the expression of the FcγIIIR (CD16) was determined on the surface of macrophages. The figure shows that IFN-γ induces macrophage B7 expression whereas IL-10 abrogates it. A reversed situation is shown for the CD16 expression; IL-10 induces the expression of the FcR whereas IFN-γ inhibits it. The same results were obtained with plastic adherent PBLC from which non-adherent cells had been removed at the start of culture (not shown).

Natural IFN-γ sources for macrophages are natural killer (NK) cells and activated T cells. Fig. 2 shows that polyclonally activated T cells upregulate macrophage B7 expression, similar to IFN-γ. Again, IL-10 acts as an antagonist much like mitogen-activated T cells block the CD16 expression by macrophages.

A role for cAMP in the phenotypic macrophage differentiation

We noted in experiments of the kind shown in Fig. 2 and 3, that activated T cells are superior to IFN-γ in suppressing the IL-10-induced CD16 expression by macrophages. Besides releasing cytokines, activated T cells engage, while forming the cellular conjugate, macrophage cell surface structures in the transduction of signals. Ligated MHC class II molecules, for instance, transmit transmembrane signals which lead to the upregulation of intracellular cAMP concentrations.[11,12] cAMP has in addition been shown to mediate the upregulation of cell surface B7 expression.[12] Results presented in Fig. 4 confirm previous studies showing the upregulation of

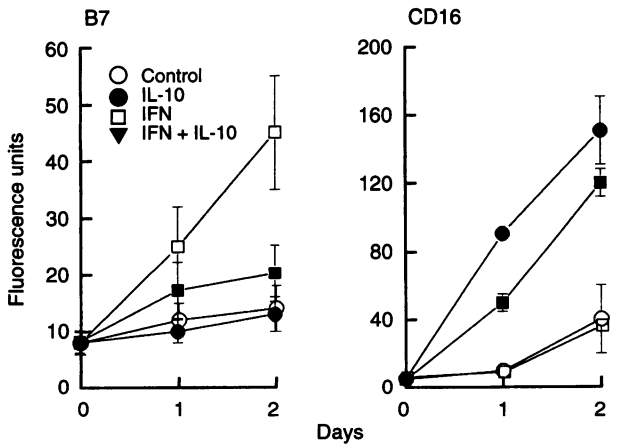

Figure 1 Antagonism between IFN-γ and Il-10 in the phenotypic macrophage differentiation. PBLC were cultured in the presence or in the absence of cytokines as indicated in the inserted box. One or 2 days later, the cells were harvested and analysed for the expression of B7 or CD16 molecules, indicated in arbitrary linear units. The data represent the averaged results from three experiments with standard deviations. IFN-γ: 500 U/ml. IL-10: 100 U/ml.

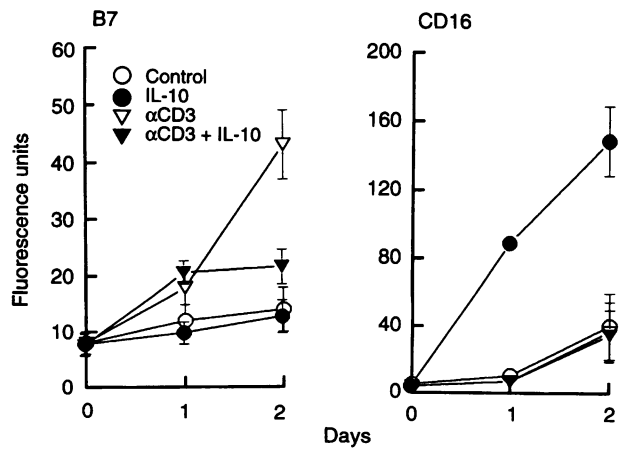

Figure 2 Activated T cells and Il-10 antagonize each others effects on the phenotypic macrophage differentiation. The experimental procedure is identical to the one described under Fig. 1 except that IFN-γ is replaced by a T-cell mitogen (1 μg/ml anti-CD3 mAb).

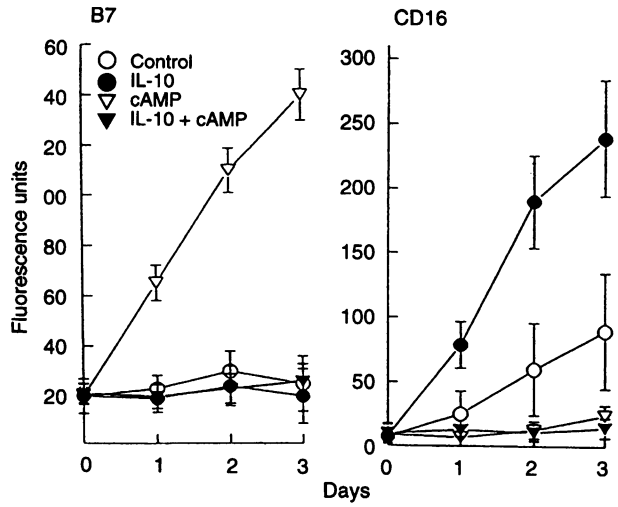

Figure 3. Distinct effects of cAMP on the expression of B7 and of CD16 on human macrophages. Human PBLC were cultured with the additions indicated in the inserted box. One to 3 days later the cells were phenotyped for B7 and CD16 expression as described under Fig. 1. The data represent the averaged results from three consecutive experiments with standard deviations. IL-10: 100 U/ml. Cell permeable dibuturyl cAMP: 1 mM.

macrophage B7 expression after treatment with cAMP and show that this pathway, too, is blocked by IL-10. The spontaneous CD16 expression, which can be blocked by anti-IL-10 mAb (not shown) as well as the CD16 expression induced with the exogenous IL-10 source, is abrogated in the presence of cAMP. Thus, while upregulating B7 expression on macrophages cAMP prevents macrophage CD16 expression.

The fact that IL-10 blocks macrophage B7 expression induced by IFN-γ or by cAMP could be taken to reflect that cAMP represents an intracellular mediator of signals transmitted by the engaged IFN-γ receptor. We see evidence in our experiments, however, that IL-10 is not simply antagonizing a cAMP function that promotes B7 expression. Rather, it

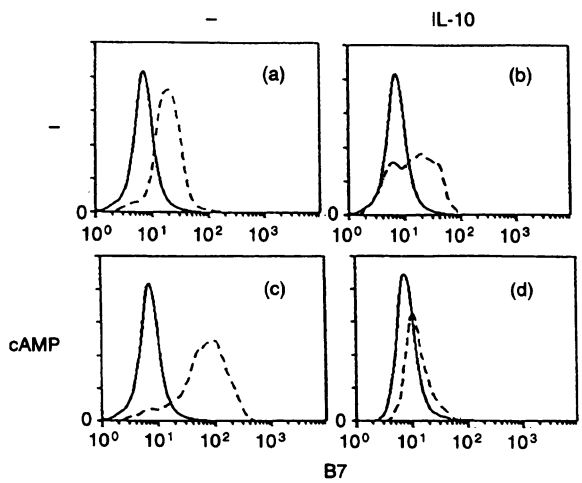

Figure 4. cAMP facilitates macrophage B7 expression in the absence of IL-10 and inhibits it in the presence of IL-10. The figure depicts individual histograms from one of the three experiments shown in the previous figure (dotted line) in comparison to the histograms of macrophage B7 expression at the culture start (continuous line).

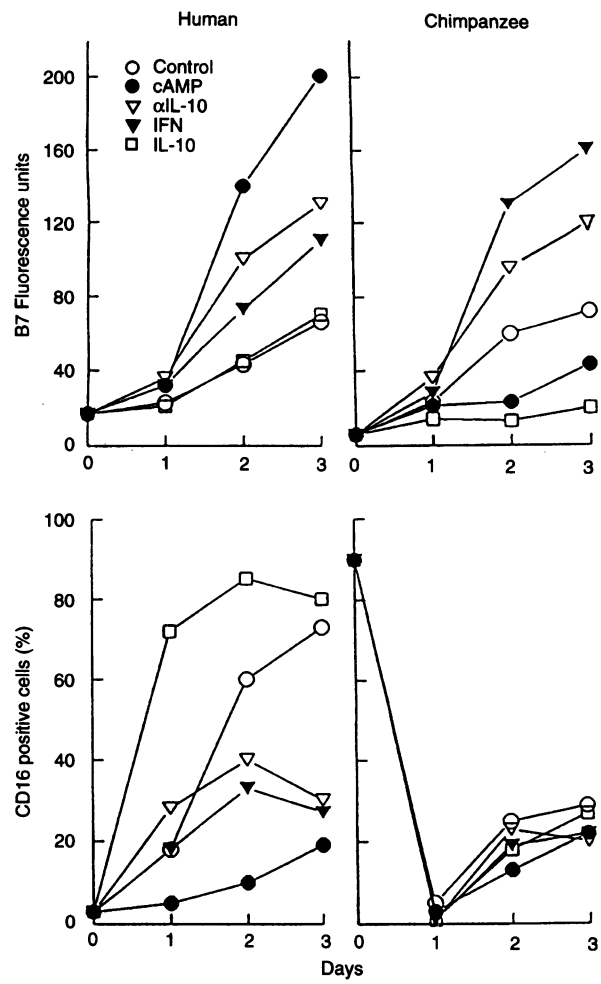

Figure 5. Humans and chimpanzees show similarities and differences in the phenotypic macrophage differentiation. Human and chimpanzee PBMC were cultured with the additions indicated in the inserted box. 1–3 days later the macrophages were phenotyped for the expression of B7 or of CD16 molecules. Anti-IL-10 mAb: 5 μg/ml. All other reagents in concentrations as before. Three chimpanzees have been examined, all essentially with the same results.

seems to reverse the cAMP function and to turn the nucleoside into a synergist of its own suppressive action on B7 expression. Fig. 4 shows the actual macrophage B7 expression histograms from one of the three experiments averaged in Fig. 4. A 3-day incubation in culture medium induces a small but distinct upregulation of B7 expression on all macrophages (Fig. 4a). A fraction of macrophages, approximately one-third, ceases to upregulate B7 expression in the presence of IL-10 (Fig. 4b). All macrophages cease in upregulating the expression of B7 when treated (Fig. 4d) with cAMP in addition to IL-10. cAMP alone induces the upregulation of B7 expression on all macrophages (Fig. 4c). Therefore, whereas in the absence of IL-10 cAMP enhances macrophage B7 expression, it inhibits it in the presence of IL-10.

Human and chimpanzee macrophages demonstrate a regulatory difference

In the course of experiments concerned with the role of macrophages in the pathogenesis of AIDS, we obtained an

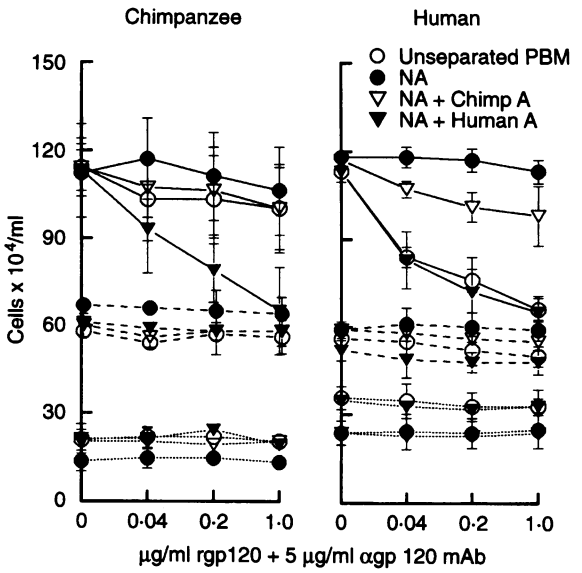

Figure 6 Lack of ADCC activity by chimpanzee macrophages. Lymphocyte-enriched (NA) human or chimpanzee lymphoid cells were cultured alone or in the presence of macrophage-enriched (A) populations of either donor in comparison to unseparated PBMC. Anti-gp120 mAb and gp120 HIV-1 envelope molecules were added as indicated. The frequency of surviving CD4 T cells (continuous line), CD8 T cells (interrupted line) or double-negative cells (dotted line) was determined two days later.

opportunity to study macrophages from chimpanzees. Chimpanzees can be infected with HIV-1. However, in contrast to humans, the virus does not seem to be able to infect, or to replicate in, chimpanzee macrophages.[13] HIV-1-infected chimpanzees do not exhibit a declining CD4 T-cell count and they do not develop AIDS.

Chimpanzees are immunologically closely related to humans; their cell surface markers are readily recognized by monoclonal antibodies raised against human cell surface structures. Measuring macrophage B7 and CD16 expression we obtained results illustrated in Fig. 5. Whereas IFN-γ induces B7 expression on macrophages from both species, cAMP induces the B7 expression on human macrophages but suppresses B7 expression on chimpanzee macrophages. In the analysis of macrophage CD16 expression, chimpanzees offered another surprise. Chimpanzee macrophages, in the developmental state of monocytes (contained in freshly prepared PBLC), express CD16 at high density but CD16 disappears rapidly from the cell surface after they had been placed into tissue culture where they turn into the resident type macrophages. CD16 expression is not reintroducible in chimpanzee macrophages with IL-10.

As previous studies in this and other laboratories identified CD16 expression as a requirement for the immune complex-mediated destruction of T cells by macrophages,[4] we wondered whether chimpanzee macrophages, bared of CD16 surface molecules, are capable of destroying antibody-reactive T cells. Specifically, we wished to know whether chimpanzee macrophages destroy T cells reactive with immune-complexed, CD4-reactive HIV-1 envelope molecules. The assay procedure, developed in this laboratory and extensively used in experiments designed to study the macrophage- and HIV-dependent deletion of CD4 T cells, measures the physical loss of immune complex-reactive T cells in tissue culture. T-cell death occurs through apoptosis and it can be blocked by masking CD95 molecules (Orlikowsky *et al.*, submitted). Fig. 6 shows that human macrophages but not chimpanzee macrophages destroy CD4 T cells in the presence of CD4-reactive immune-complexed HIV-1 envelope molecules.

DISCUSSION

The presented data identify IFN-γ and IL-10 as antagonists in the induction of macrophage differentiation. The two cytokines provide the macrophage with a differentiation choice, endowing them either with immune-supportive or with immune-destructive capacities.

The dichotomy of the two differentiation pathways is modulated by the presence of intracellular cAMP concentrations. This nucleoside inhibits macrophage CD16 expression. In regulating macrophage B7 expression, cAMP seems to have an option; in the absence of IL-10, the nucleoside facilitates B7 expression whereas in the presence of IL-10, it inhibits it. It is conceivable that cAMP induces macrophage B7 expression by amplifying subthreshold IFN-γ stimulation and that it inhibits macrophage B7 expression by amplifying IL-10 stimulation. The differential effects of cAMP on the B7 expression on human and chimpanzee macrophages could thereby be explained by a relative IFN-γ predominance in humans and a relative IL-10 predominance in chimpanzees. The high monocyte CD16 expression lends support to the assumption of IL-10 predominance in the primate. Verification of an IL-10 predominance in the chimpanzee would be of interest in view of the observation that IL-10 inhibits HIV-1 replication in macrophages but not in T cells;[14–15] in the chimpanzee, HIV-1 is reported to replicate in T cells but not in macrophages.[13]

Whereas IFN-γ and IL-10 direct macrophages to enter distinct differentiation pathways, the presented data also demonstrate that in the presence of IL-10 and cAMP, macrophages fail to enter either differentiation pathway. Current experiments reveal that macrophages treated with IL-10 and cAMP lack the ability to present antigen to T cells, lack the ability to destroy antibody-reactive T cells, and are resistant to activation-induced cell death. The results suggest the possibility that the massive upregulation of intracellular cAMP levels enables the macrophage to enter, in the presence of IL-10, a state of paralysis.

Our findings concerning the phenotypic macrophage differentiation are consistent with previous observations that IFN-γ enhances the macrophage B7 expression and that this response can be blocked with IL-10.[5,8] Regarding the enhanced CD16 expression on IL-10-treated macrophages our findings are in apparent conflict with the literature[16,17] which demonstrates that IL-10 does not substantially upregulate CD16 expression on monocytes. In these studies, monocytes were cultured on teflon-fluorinated-ethylen-propylen bottles to prevent their attachment to the culture plate and their conversion into macrophages, whereas in our experiment monocytes were allowed to attach to plastic surfaces and to become macrophages. It is conceivable that differentiation into macrophages is a prerequisite for monocytes to respond to IL-10 exposure with the upregulation of CD16.

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