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TGF- β 1 pretreatment impairs the allostimulatory function of human bone marrow-derived antigen-presenting cells for both naive and primed T cells

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Abstract

Transforming growth factor-beta (TGF- β) exhibits strong antiproliferative effects upon lymphocytes and inhibits many of the effector functions of activated immune cells. However, its influence on the inductive phase of immune responses, and in particular its effect on antigen-presenting cells (APC), has not been well studied. In this investigation, we examined the influence of human TGF- β 1 on the antigen-presenting function of human bone marrow (BM)-derived APC propagated in liquid culture for 11–17 days in response to granulocyte/macrophage colony-stimulating factor (GM-CSF). These cells were predominantly macrophages, accompanied by a minor population of dendritic cells. TGF- β 1 had no effect upon the allostimulatory function of vertebral body whole BM cells cultured for 3–5 days in GM-CSF. However, it markedly reduced the allostimulatory capacity of BM-derived APC exposed to the cytokine for the last 3 days of culture. This inhibitory action could not be ascribed to cytokine ‘carry-over’, or to any consistent changes in the expression of cell surface molecules implicated in antigen presentation (HLA-DR), intercellular adhesion (ICAM-1; CD54), or costimulatory activity (B7-1; CD80). Mechanisms that may underlie the inhibitory action of TGF- β on APC function and the immunologic and possible clinical implications of the findings are discussed.

Introduction

Transforming growth factor-beta (TGF- β) is a growth inhibitory cytokine that exerts multiple actions on most cells. It plays an important role in the regulation of immune reactivity, wound healing, and tissue development.^{1,2} Three isoforms of TGF- β have been identified, each encoded by its own gene, and with a sequence homology of 70–80%.¹ The nature of its effects depends on several parameters, including cell type, differentiation, local environment, and the presence of other growth factors and cytokines. The immunomodulatory activities of TGF- β have been extensively described.^{1–6} It has strong antiproliferative effects on lymphocytes,^{6–9} and downregulates interferon-gamma (IFN- γ)-induced cell surface major histocompatibility complex (MHC) class II antigen expression.¹⁰ TGF- β decreases the expression of many cytokines including IFN- γ , tumor necrosis factor-alpha and interleukins IL-1, IL-2 and IL-3. In contrast, it increases production of IL-1 receptor antagonist,^{2,4–6,11} an observation consistent with its anti-inflammatory properties. TGF- β inhibits IL-2 receptor expression^{4,5} and the effector functions of activated cells, such as the cytolytic activity of NK (natural killer) and LAK (lymphokine-activated killer)

cells.^{7,9} It deactivates differentiated macrophages, suppressing the respiratory burst and H₂O₂ release.¹² However, its effects on the inductive arm of the immune response (i.e. antigen presentation and lymphocyte activation) have not been well characterized.

The bone marrow (BM) contains a number of cells with the potential to develop into or serve as antigen-presenting cells (APC).^{13,14} These cells are of interest both in the context of BM transplantation and rejection, and with regard to the augmentation of donor leukocyte chimerism by BM infusion at the time of solid organ transplantation.¹⁵ Among the potential APC resident in the BM are B lymphocytes, monocytes and dendritic cells. It has been postulated that donor BM-derived APC could play a key role in the establishment of tolerance following organ transplantation.^{16–18} Furthermore, recognition that the relative tolerogenicity of the transplanted liver could be related to its rich endowment of BM-derived leukocytes¹⁶ has led to clinical trials using BM to augment donor cell chimerism in solid organ transplantation.¹⁵ BM is a rich source from which large numbers of APC may be propagated. The maturation and function of these cells is markedly affected by the cytokine milieu. Thus, it has recently been shown that granulocyte/macrophage colony-stimulating factor (GM-CSF), either alone or in combination with other cytokines, promotes the maturation and development both of macrophages and dendritic cells.^{19–22}

Objective

The objective was to explore the influence of TGF- β 1 on the growth and allostimulatory activity of cells present in unmodified human vertebral body BM, and in APC-enriched populations grown in response to recombinant (r) human (h) GM-CSF. The cells were tested using both naive and primed T cells as responders and characterized with a panel of monoclonal antibodies (mAbs) directed against key cell surface phenotypic and functional markers.

Materials and methods

Preparation, culture and treatment of BM cells

Permission to use BM from human cadaveric organ donors for research purposes was obtained from the donor family as per University of Pittsburgh Medical Center Internal Review Board protocol. Thoracolumbar vertebral bodies were processed as described elsewhere.¹⁵ BM cell suspensions were prepared, and erythrocytes lysed with sterile water for 3 s. Lysis was quenched with an appropriate volume of 10 $\times$ Hanks' Balanced Salt Solution (HBSS; Gibco BRL, Grand Island, NY) to render the final suspension isotonic. The cells were then washed twice with HBSS. Viability was confirmed by trypan blue dye exclusion. The cells were resuspended in RPMI-1640 (Gibco BRL) supplemented with 5% (v/v) human AB serum (Nalgene, Miami, FL), nonessential amino acids, sodium pyruvate, L-glutamine, penicillin–streptomycin, 2-mercaptoethanol (all from Gibco BRL), and 0.4 ng/ml rhGM-CSF (R&D Systems, Minneapolis, MN). Two $\times 10^6$ cells were placed in each well of a 24 well plate in a volume of 2 ml. The cultures were maintained at 37°C in humidified 5% CO₂ in air, and fed every other day by aspirating half of the supernatant and then replenishing the wells with an equivalent volume of fresh, GM-CSF-supplemented medium. To enrich for APC, the plate were swirled gently prior to aspiration to remove nonadherent granulocytes, without dislodging clusters of presumptive dendritic cell progenitors attached loosely to monolayers of plastic-adherent macrophages.^{19,22} For cultured whole BM, no attempt was made to remove nonadherent cells over the 3–5 day culture period. The cultures were inspected daily and, when growing clusters of cells were abundant (at 8–14 days), subcultures were performed.

The mature 25 kDa dimer of rhTGF- β 1 (R&D Systems; 0.6 ng/ml) was added to selected plates. The cells were cultured for an additional 3 days and then harvested by pipetting. They were washed three times in a large excess of HBSS to remove any free contaminating cytokines. Decanted supernatants were saved for later analysis. Harvested cells, which consisted of those easily dislodged by pipetting, were used in functional assays and immunophenotypic analysis. Cytospin preparations were stained with Giemsa to demonstrate cell morphology.

mAbs

Mouse mAbs to the following human cell surface antigens were used: Leu-4 (T cell; CD3 epsilon chain), Leu-3a (T helper/inducer; CD4), Leu-2a (T suppressor/cytotoxic; CD8), Leu-19 (NK cell; CD56), Leu-12 (B cell; CD19), Leu-M3 (macrophage; CD14), H1e-1 (leukocyte common antigen, LCA; CD45), HLA-DR (MHC class II), CD11a (LFA-1 α), Leu-15 (CR₃; CD11b), Leu-M5 (CR₄; CD11c), CD18 (LFA-1 β), Leu-5b (LFA-2; CD2), CD80 (BB1/B7) (all Becton Dickinson, San Jose, CA); T6-RD1 (CD1a) (Coulter Immunology, Hialeah, FL); CD54 (ICAM-1) (PharMingen, San Diego, CA).

Staining of cell surface antigens

Immunophenotypic analysis was performed by cytofluorography, using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Cells harvested from human BM cultures were stained with the appropriate mouse IgG isotypic subclass controls to detect the presence of nonspecific antibody staining. Two $\times 10^5$ cells per sample were first blocked with 10% (v/v) normal goat serum (Vector Labs, Burlingame, CA) in HBSS containing 0.1 % bovine serum albumin (BSA; Sigma, St Louis, MO) for 30 min at 4°C. Cells were then washed once with 0.1 % BSA/HBSS and centrifuged at $400 \times g$, 4°C for 6 min. Supernatants were decanted and the cells resuspended in 100 μ l. They were then stained with FITC- or phycoerythrin-conjugated mouse mAbs for 45 min 4°C. Excess antibody was removed by washing twice in 0.1 % BSA/HBSS. Samples were fixed in 1% paraformaldehyde (Sigma) prior to analysis. Typically, 5000 events were analyzed from each sample. Cells were analyzed by gating on the large, granular population.

Functional assays

The allostimulatory activity of whole BM cells and APC-enriched BM-derived populations was tested in primary mixed lymphocyte reactions (MLR) and pooled primed lymphocyte tests (pLT). Cultured cells γ -irradiated with 20 Gy served as stimulators. Cryopreserved donor-derived splenocytes were irradiated to serve as positive controls. For use as responders in MLR, peripheral blood lymphocytes (PBL) were isolated from heparinized blood of normal healthy donors by centrifugation over Ficoll-Hypaque. Six-day unidirectional MLR cultures were established with a fixed number of PBL responders (1×10^5 cells per well) and varying numbers of irradiated stimulators. Triplicate cultures were performed in 96 well plates, in 200 μ l of tissue culture medium supplemented with 5% (v/v) human AB serum, at 37°C, in a humidified atmosphere of 5% CO₂ in air. During the final 18 h of incubation, each culture was labeled with 1 μ Ci of [³H]thymidine (ICN Radiochemicals, Costa Mesa, CA). Cells were harvested onto glass fiber disks using a multiple cell harvester, and the degree of thymidine incorporation was determined in a liquid scintillation counter. To test for TGF- β 1 'carry over', supernatants from washed cells were added to lymphocytes stimulated by donor spleen cells at the initiation of culture. Thymidine uptake was measured during the last 18 h of culture.

For the PLT, PBL from healthy donors were primed by a large, pooled panel of irradiated cells of different HLA types (pooled PLT reagent). Alloreactive lymphocytes served as responders in a secondary assay using irradiated BM-derived APC as stimulators. The

number of responders was fixed at 2.5×10^4 cells per well, while the number of stimulators was varied. Cultures were performed in triplicate (200 μ l per well), in 96 well plates, for 3 days and [3 H]thymidine uptake was assessed as described above.

Statistics

Statistical analysis was performed using paired *t*-tests on Statview 4.0.1 software (Abacus Concepts, Berkeley, CA). A *P* value < 0.05 was considered significant.

Results

Effects of TGF- β 1 on the growth and morphologic appearance of GM-CSF-stimulated BM-derived APC

A total of 4.8×10^7 whole BM cells were placed initially in each culture plate (2×10^6 cells per well in a 24 well plate). After extended culture in GM-CSF for 11–17 days, typically $0.75\text{--}1 \times 10^6$ cells per plate were recovered from cultures enriched for APC. When TGF- β 1 was added for the last 3 days of culture, similar number of cells were recovered, demonstrating that TGF- β 1 had no adverse effect on the viability of the cultured cells. Geisma-stained cytocentrifuge preparations revealed that the APC-enriched population resembled cells reported previously in GM-CSF-stimulated liquid cultures.^{20,21} The morphologic appearance of the cells (approx. 60–70% macrophages and 20–30% dendritic cells) was not affected by 3 days of exposure to TGF- β .

Effects of TGF- β 1 on the phenotype of cultured whole BM and GM-CSF-stimulated BM-derived APC

For purposes of comparison with cultured cells, flow cytometry was performed on samples of freshly isolated BM cells from seven cadaveric donors. These cells expressed the leukocyte common antigen CD45. The incidence of the T cell markers CD3, CD4 and CD8 was low, as was that of the B cell marker, CD19, and HLA-DR (MHC class II). There was also a low incidence of cells expressing the monocyte marker CD14; NK cell (CD56) and dendritic cell (CD1a) markers were also identified. The adhesion molecules CD11a, CD11b, CD18 and CD54 (ICAM-1) were all expressed on up to 50% of the cells. CD2 and CD11c were not detected. B7-1 (CD80) expression was very low. After 3–5 days of culture in GM-CSF, there were no significant differences in the percentages of whole BM cells staining for each of these markers either between samples or in comparison to fresh BM. In addition, cells incubated in GM-CSF, and with TGF- β 1 added for the last 3 days of culture, exhibited a phenotype similar to that of cells grown in GM-CSF alone (data not shown).

The results of FACScan analysis of the APC-enriched BM-derived cells are presented in Table 1. The putative APC showed a comparatively high incidence of CD45⁺ and HLA-DR⁺ cells. As anticipated from the morphologic appearance of the cells, B cell, T cell and NK cell markers were very low. The incidence of cells expressing the monocyte marker CD14, however, was relatively high, and staining was comparatively bright compared to cells from whole bone marrow. Both the incidence and staining intensity of cells expressing the dendritic cell marker CD1a was low. The adhesion and costimulatory molecule CD54, however, was present on most of the APC population. B7-1 was found on only a small proportion of the cells. Similar observations on GM-CSF-stimulated human blood-derived cells have recently been reported.^{20,21} As with whole BM cells, the addition of TGF- β 1 during the final 3 days of culture did not affect the cell surface phenotype of the APC-enriched population, although there was a trend towards lower percentages of cells expressing CD1a and B7-1.

Allostimulatory activity of BM-derived APC

Cultures of whole BM cells and those enriched for APC were tested as stimulators in one-way primary MLR (Figure 1). Donor splenocytes from cryopreserved cell suspensions were used as control allogeneic stimulators. As shown in Figure 1, whole BM cells were poor stimulators of allogeneic T cells when compared to splenocytes. In contrast, GM-CSF-stimulated BM-derived APC were consistently three to five times more potent than spleen cells at inducing T cell proliferation. At low relative stimulator cell numbers, at which spleen cells could no longer induce T cell proliferation, the APC exhibited marked allostimulatory activity.

The effects of TGF- β 1 on the allostimulatory activity of BM-derived APC in primary MLR

When TGF- β 1 was added for the last 3 days to 3–5 day cultures of whole BM, no significant effect was observed on the already low allostimulatory activity of these cells (Figure 2). However, when TGF- β 1 was added to the APC-enriched population during the last 3 days of 11–17 day cultures, a pronounced inhibitory effect on their potent allostimulatory function was observed (Figure 3A). The effect was not seen if the TGF- β 1 was withheld until the last 24 h of culture (data not shown).

Since it was conceivable that TGF- β 1 ‘carried over’ with pretreated stimulator cells could have contributed to the inhibitory effect observed, supernatants from TGF- β 1-treated cultures were tested for inhibitory activity in MLR. They were found to have no effect on T cell proliferation in response to donor spleen cells (data not shown). This implies that the loss of allostimulatory activity was a result of a direct effect of TGF- β 1 on the APC, rather than to a ‘carry over’ effect of TGF- β 1 on the responder T cells.

Effects of TGF- β 1 on the stimulatory activity of APC for primed T lymphocytes

The APC-enriched GM-CSF-stimulated cells (11–17 days of culture) were tested for their ability to stimulate T cells that had been previously primed by a pooled panel of HLA antigens. As in the primary MLR, TGF- β 1 reduced the allostimulatory capacity of the APC, again by about 50% (Figure 3B).

Discussion

There have been few reports of the effects of TGF- β on the function of APC. It has been shown, however, that TGF- β inhibits antigen presentation by peripheral blood mononuclear cells and cultured Langerhans cells, but not by freshly isolated Langerhans cells.²³ TGF- β has been implicated in endowing macrophages with the capacity to induce antigen-specific immune deviation,²⁴ whereas it did not suppress tumor immunity conferred by epidermal APC.²⁵

In the present experiments, the allostimulatory capacity of whole BM cells was not affected by TGF- β 1. As its allostimulatory activity was initially much lower than that of the GM-CSF-stimulated APC-enriched population, whole BM may have contained insufficient numbers of TGF- β responsive APC to exhibit an effect. The antigen-presenting capability of the latter cells, however, appears to be markedly diminished by exposure to TGF- β . The mechanism of this action is unclear. Although the overall viability of the cells was not influenced, the quantities of specific cell types may have been altered by TGF- β 1, even though no significant differences were detected by flow cytometric analysis. Relatively small changes in the numbers of potent APC, such as dendritic cells, which were not detected by flow cytometry, may have profound effects on the MLR. Although there was a trend towards fewer cells expressing the dendritic cell marker CD1a with TGF- β 1 treatment, this was not statistically significant. Mature dendritic cells are known to be potent

stimulators of naive T cells.²⁶ Therefore, relatively small changes in their numbers may have profound effects on the MLR. Macrophages and activated B cells also possess the ability to stimulate T cells, although to a much lesser degree than dendritic cells. The immunostimulatory activity of each of these cells correlates with the level of cell surface expression of MHC class II and costimulatory molecules.^{13,14} Their comparative accessory function is not so strikingly different in the stimulation of primed T cell secondary allogeneic responses, probably due to a less stringent requirement for MHC class II involvement.²⁷ The effects of TGF- β 1 on the APC used in the PLT were nevertheless quite impressive. As suggested by the present results, TGF- β 1 may exert its influence by means other than altering the expression of MHC class II. Since TGF- β is known to inhibit cytokine production, it may attenuate the function of APC by suppression of one or more cytokine signals.

Costimulatory molecule expression was not changed significantly by TGF- β 1 when considering the APC as a whole, except for a trend towards lower B7-1 expression. This may have contributed, in part, to the loss of allostimulatory capacity of the APC. However, detailed analysis of individual cell types was not performed. Conceivably, TGF- β may increase costimulatory molecule expression on some cell types, while simultaneously decreasing expression of the same molecules on others. Such an effect could disrupt the second signal necessary for activating T cells.

TGF- β can induce autocrine secretion.^{1,3,6} However in our experiments, supernatants from washed APC did not affect the proliferation assays. Even so, the addition of APC secreting a continuously low level of TGF- β , or the presence of a latent form of TGF- β which later becomes activated in prolonged cultures, cannot be ruled out. TGF- β may have an effect on feedback received by the APC upon interaction with T cells. A third and a fourth signal in antigen presentation and T cell activation have been proposed.²⁸ Thus, T cell interaction with MHC class II molecules may activate APC, resulting in improved costimulatory activity. T cell secreted cytokines, such as IL-4, may also affect APC surface molecule expression and function. TGF- β may have an impact on signal transduction pathways involved in the processing of these third and fourth signals.

The ability of TGF- β (at concentrations substantially below those encountered in inflammatory conditions²⁹) to inhibit the antigen-presenting capacity of accessory cells may have far-reaching clinical implications. It has been suggested that stimulation of TGF- β production by T cells may, in part, underlie the therapeutic efficacy of cyclosporin A.^{2,30} TGF- β prolongs heart allograft and pancreatic islet xenograft survival in rats.^{31,32} It also reduces the severity of experimentally induced allergic encephalomyelitis³³ and arthritis.³⁴ Moreover, it may be responsible for the postulated 'veto' function (inactivation/deletion of cytotoxic T cell precursors) of a donor BM-derived population that can promote the induction of transplant tolerance in primates.³⁵ Augmentation of the acceptance of solid organ transplants with BM infusion is being attempted in several centers.^{15,16,36} Cytokines such as TGF- β produced constitutively or as the result of gene transfer³⁷ may influence significantly the allostimulatory activity of the transplanted cells and the host-donor immunologic interaction. Further studies, particularly observations in animal models, will indicate the potential clinical significance of TGF- β for the therapy of allograft rejection.

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References

1. Derynck, R. Transforming growth factor-beta. In: Thomson, AW., editor. The cytokine handbook. second edition. San Diego: Academic Press; 1994. p. 319-342.
2. Roberts AB, Sporn MB. Physiological actions and clinical applications of transforming growth factor- β (TGF- β). *Growth Factors*. 1993; 8:1-9. [PubMed: 8448037]
3. Wahl SM, McCartney-Francis N, Mergenhagen SE. Inflammatory and immunomodulatory roles of TGF-beta. *Immunol Today*. 1989; 10:258-261. [PubMed: 2478145]
4. Ruscetti F, Varesio L, Ochoa A, Ortaldo J. Pleiotropic effects of transforming growth factor-beta on cells of the immune system. *Ann NY Acad Sci*. 1993; 685:488-500. [PubMed: 8363258]
5. Fontana A, Constan DB, Frei K, Malipiero U, Pfister HW. Modulation of the immune response by transforming growth factor beta. *Int Arch Allergy Immunol*. 1992; 99:1-7. [PubMed: 1483057]
6. Kehrl JH. Transforming growth factor-beta: an important mediator of immunoregulation. *Int J Cell Cloning*. 1991; 9:438-450. [PubMed: 1955735]
7. Rook AH, Kehrl JH, Wakefield LM, et al. Effects of transforming growth factor beta on the functions of natural killer cells; depressed cytolytic activity and blunting of interferon responsiveness. *J Immunol*. 1986; 136:3916-3920. [PubMed: 2871107]
8. Kehrl JH, Taylor A, Kim SJ, Fauci AS. Transforming growth factor- β is a potent negative regulator of human lymphocytes. *Ann NY Acad Sci*. 1991; 628:345-353. [PubMed: 1648884]
9. Smyth MJ, Strobl SL, Young HA, Ortaldo JR, Ochoa AC. Regulation of lymphokine-activated killer activity and pre-forming protein gene expression in human peripheral blood CD8⁺ T lymphocytes. Inhibition by transforming growth factor-beta. *J Immunol*. 1991; 146:3289-3297. [PubMed: 1827481]
10. Czarniecki CW, Chiu HH, Wong GH, McCabe SM, Palladino MA. Transforming growth factor-beta 1 modulates the expression of class II histocompatibility antigens on human cells. *J Immunol*. 1988; 140:4217-4223. [PubMed: 3131428]
11. Espevik T, Figari IS, Shalaby MR, et al. Inhibition of cytokine production by cyclosporin A and transforming growth factor beta. *J Exp Med*. 1987; 166:571-576. [PubMed: 3110354]
12. Tsunawaki S, Sporn M, Ding A, Nathan C. Deactivation of macrophages by transforming growth factor-beta. *Nature*. 1988; 334:260-262. [PubMed: 3041283]
13. McCluskey, J.; Blok, R.; Brooks, A.; Chen, W.; Kanost, D.; Kjer-Nielsen, L. The biology of antigen processing and presentation. In: McCluskey, J., editor. Antigen processing and recognition. Boca Raton: CRC Press; 1991. p. 1-54.
14. Unanue, ER. Macrophages, antigen-presenting cells, and the phenomena of antigen handling and presentation. In: Paul, WE., editor. Fundamental immunology. New York: Raven Press; 1993. p. 111-144.
15. Fontes P, Rao AS, Demetris AJ, et al. Bone marrow augmentation of donor-cell chimerism in kidney, liver, heart, and pancreas islet transplantation. *Lancet*. 1994; 344:151-155. [PubMed: 7912764]
16. Starzl TE, Demetris AJ, Trucco M, et al. Cell migration and chimerism after whole-organ transplantation: the basis of graft acceptance. *Hepatology*. 1993; 17:1127. [PubMed: 8514264]
17. Steinman RM, Inaba K, Austyn JM. Donor-derived chimerism in recipients of organ transplants. *Hepatology*. 1993; 17:1153-1156. [PubMed: 8514265]
18. Thomson AW, Lu L, Murase N, Demetris AJ, Rao AS, Starzl TE. Microchimerism, dendritic cell progenitors and transplantation tolerance. *Stem Cells*. 1995; 13:622-639. [PubMed: 8590864]
19. Inaba K, Inaba M, Romani N, et al. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med*. 1992; 176:1693-1702. [PubMed: 1460426]
20. Caux C, Dezutter-Dambuyant D, Schmitt D, Banchereau J. GM-CSF and TNF- α cooperate in the generation of dendritic Langerhans cells. *Nature*. 1992; 360:258-261. [PubMed: 1279441]
21. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α . *J Exp Med*. 1994; 179:1109-1118. [PubMed: 8145033]

22. Lu L, Woo J, Rao AS, et al. Propagation of dendritic cell progenitors from normal mouse liver using granulocyte/macrophage colony-stimulating factor and their maturational development in the presence of type-1 collagen. *J Exp Med*. 1994; 179:1823–1834. [PubMed: 8195710]
23. Demidem A, Taylor JR, Grammer SF, Streilein JW. Comparison of effects of transforming growth factor-beta and cyclosporin A on antigen-presenting cells of blood and epidermis. *J Invest Dermatol*. 1991; 96:401–407. [PubMed: 1826121]
24. Wilbanks GA, Streilein JW. Fluids from immune privileged sites endow macrophages with the capacity to induce antigen-specific immune deviation via a mechanism involving transforming growth factor- β . *Eur J Immunol*. 1992; 22:1031–1036. [PubMed: 1551403]
25. Grabbe S, Bruvers S, Granstein RD. Interleukin 1 α but not transforming growth factor β inhibits tumor antigen presentation by epidermal antigen-presenting cells. *J Invest Dermatol*. 1994; 102:67–73. [PubMed: 8288913]
26. Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol*. 1991; 9:271–296. [PubMed: 1910679]
27. Thomas R, Davis LS, Lipsky PE. Comparative accessory cell function of human peripheral blood dendritic cells and monocytes. *J Immunol*. 1993; 151:6840–6852. [PubMed: 8258694]
28. Hart, DNJ.; Calder, VL. Human dendritic cells: function and cytokine production. In: Bruijnzeel-Koomen, CA.; Hoefsmit, FC., editors. *Immunopharmacology of macrophages and other antigen-presenting cells*. San Diego: Academic Press; 1994. p. 63-91.
29. Fava R, Olsen N, Keski-Oja J, Moses H, Pincus T. Active and latent forms of TGF-beta activity in synovial effusions. *J Exp Med*. 1989; 169:291–296. [PubMed: 2909658]
30. Li B, Sehajpal PK, Khanna A, et al. Differential regulation of transforming growth factor β and interleukin 2 genes in human T cells: demonstration by usage of novel competitor DNA constructs in the quantitative polymerase chain reaction. *J Exp Med*. 1991; 174:1259–1262. [PubMed: 1682412]
31. Raju GP, Belland SE, Eisen HJ. Prolongation of cardiac allograft survival with transforming growth factor- β 1 in rats. *Transplantation*. 1994; 58:392–396. [PubMed: 8053069]
32. Carel JC, Sheehan KC, Schreiber RD, Lacy PE. Prevention of rejection of transforming growth factor beta-treated rat-to-mouse islet xenografts by monoclonal antibody to tumor necrosis factor. *Transplantation*. 1993; 55:456–458. [PubMed: 8434409]
33. Kuruvilla AP, Shah R, Hochwald GM, Liggitt HD, Palladino MA, Thorbecke GJ. Protective effect of transforming growth factor beta 1 on experimental autoimmune diseases in mice. *Proc Natl Acad Sci USA*. 1991; 88:2918–2921. [PubMed: 2011600]
34. Brandes ME, Allen JB, Ogawa Y, Wahl SM. Transforming growth factor beta 1 suppresses acute and chronic arthritis in experimental animals. *J Clin Invest*. 1991; 87:1108–1113. [PubMed: 1999490]
35. Verbanac KM, Carver FM, Haisch CE, Thomas JM. A role for transforming growth factor-beta in the veto mechanism in transplant tolerance. *Transplantation*. 1994; 57:893–900. [PubMed: 8154038]
36. Barber WH, Mankin JA, Laskow DA, et al. Long term results of a controlled prospective study with transfusion of donor-specific bone marrow in 57 cadaveric renal allograft recipients. *Transplantation*. 1991; 51:70–75. [PubMed: 1987708]
37. Qin L, Chavin KD, Ding Y, et al. Multiple vectors effectively achieve gene transfer in a murine cardiac transplantation model. *Transplantation*. 1995; 59:809–816. [PubMed: 7701573]

Figure 1.

Six day primary MLR showing allogeneic T lymphocyte activation in response to three different stimulator cell populations. Eleven to 17 day GM-CSF-stimulated APC were better stimulators of naive allogeneic T cells than spleen cells from the same donor. In contrast, 3–5 day GM-CSF-stimulated whole BM cells were poor stimulators of the MLR. Vertical bars denote standard deviations of the mean. Results are representative of four separate experiments.

Figure 2.

Primary MLR demonstrating the allostimulatory activity of GM-CSF-stimulated whole BM cells (3–5 day cultures) pretreated with TGF-β1. TGF-β1 treatment of the cells did not affect their allostimulatory activity. Vertical bars denote standard deviations of the mean. Results are representative of at least three experiments.

Figure 3.

(A) MLR demonstrating the effect of TGF- β 1 pretreatment of BM-derived APC (11–17 day cultures) on their allostimulatory activity in primary MLR. TGF- β 1 treatment for the last 3 days of culture reduced the subsequent allostimulatory activity of the APC. Standard deviations are displayed as vertical bars. (B) PLT showing the effects of TGF- β 1 pretreatment on the stimulatory activity of APC cultured with primed T lymphocytes in a secondary response. TGF- β 1 reduced the allostimulation by the APC. Results are representative of at least three experiments.

Table 1Expression of cell surface antigens by TGF- β 1-treated BM-derived APC

Antigen	GM-CSF only		GM-CSF + TGF- β 1	
	% positive ^a	MFI ^b	% positive ^a	MFI ^b
CD45	57.7 (46–70)	33.8	63.6 (29–64)	46.0
HLA-DR	48.9 (29–80)	136.6	42.3 (38–64)	135.3
CD3	3.5 (0–7.1)	9.1	2.2 (1.7–7.1)	7.5
CD19	0.6 (0.1–3.5)	6.8	2.3 (0–2.4)	12.0
CD14	62.7 (53–79)	140.5	61.4 (57–65)	113.0
CD1a	16.9 (5.8–43)	12.8	8.4 (7.2–22)	11.6
CD54	89.0 (84–92)	168.8	83.8 (77–90)	184.0
B7-1	11.6 (0–16)	19.1	2.5 (2.3–11)	19.1

^aValues shown are median values of the percentage of positively staining cells determined in cultures from 3–7 bone marrow donors. Ranges are shown in parentheses.

^bMean fluorescence intensity of cells.