

Platelet-derived Growth Factor Is a Potent Biologic Response Modifier of T Cells

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Summary

Freshly isolated lymph node (LN) cells cultured in serum-containing medium were restricted to produce primarily interleukin 2 (IL-2) subsequent to T cell activation. Only minimal amounts of IL-4, IL-5, or interferon γ (IFN- γ) were produced under these conditions. Similar populations of LN cells cultured in serum-free medium were able to produce a variety of lymphokines after T cell activation, with the relative quantities of each species being dependent upon the lymphoid organ source of the lymphocytes. A similar relationship in the patterns of lymphokines produced by activated T cell hybridomas maintained under serum-free conditions was also observed, whereas activation in serum-supplemented media resulted in a predominant restriction to the secretion of IL-2. Additional studies determined that the entity in serum responsible for restricting T cell function in vitro was platelet-derived growth factor (PDGF). The PDGF-BB isoform was established to be the most active in the regulation of T cell function, enhancing IL-2 while depressing the production of IL-4, IL-5, and IFN- γ at concentrations below 1 ng/ml. PDGF-AB was also found to be quite active, however, this isoform of PDGF was incapable of influencing IFN- γ production at the concentrations tested. PDGF-AA was very weakly active. It therefore appears that PDGF, acting primarily through a β receptor subunit (either α/β - or β/β -type receptors) is able to influence profoundly the behavior of T cells, with some of its modulatory effects exhibiting isoform specificity. This is reflected by an enhancement in the production of IL-2, while simultaneously depressing the secretion of IL-4, IL-5, and IFN- γ (PDGF-BB only) after T cell activation. Kinetic studies, where cell supernatants were analyzed both 24 and 48 h after T cell activation, suggested that "desensitization" to PDGF influences can occur naturally in vitro. Those species of lymphokines that were inhibited by PDGF over the first 24 h after activation could be produced at normal levels over the subsequent 24-h period. Finally, lymphokines maintained in the presence of PDGF-BB for greater than 24 h before their activation lost sensitivity to this growth factor. These cells regained responsiveness to PDGF after an additional incubation period in PDGF-free medium. Collectively, our data imply that the pattern of T cell lymphokines produced, plus the kinetics of their production after activation, are being controlled by the potent serum growth factor PDGF. These original observations have important implications to our understanding of T cell function under normal, altered, or pathologic conditions. Our data indicate that serum-supplemented culture medium, or any procedure where an exogenous source of PDGF is present, will significantly influence T cell behavior. These effects need to be considered when interpreting the results of experiments conducted in vitro.

No cell lives in an environment that is isolated from substances capable of modifying its behavior. Consequently, to fully understand the changes in growth, differentiation, and metabolism, which result from a cell's encounter with a defined stimulus, requires consideration of all factors that are influencing the cell, at or near the time of its stimulation. Mature T cells are not exempt from these basic rules of cell biology since the nature of their behavior, subsequent to antigenic stimulation, can be controlled by a variety of exoge-

nous influences. These include the arachidonic acid metabolites (1, 2), steroid and polypeptide hormones (3-7), plus any other substances or physical properties able to quantitatively or qualitatively alter cellular responsiveness (e.g., temperature, available nutrients, etc.).

Much of our present knowledge about T cells and the mechanisms that govern their behavior has evolved from studies conducted in vitro. Differences among the responses elicited by cells that are isolated from a particular lymphoid tissue

and placed into an *in vitro* environment, in the presence or absence of a given stimulant, are taken to represent the inherent responsiveness of the T cell. The failure of T cells within the unstimulated "control" population to express a particular response is generally considered to reflect the absence of any inherent changes within these cells on the genes or gene products encoding or controlling that response.

The complexity of regulatory control that exists over most cellular genes, with numerous individual influences able to alter such events as the rate and duration of transcription, translation, posttranslational modifications, and secretory processes, indicates that it may be unrealistic not to consider that some relevant influences are being exerted on populations of T cells taken from *in vivo* lymphoid tissue environments and placed *in vitro*. The presence of these influences might only become evident after T cell activation, since stimulation may be necessary to elicit the desired changes in cellular behavior. A simple example of this concept can be demonstrated by the depressive influences on IL-2 or IFN- γ production on resting T cells exposed to either glucocorticoids (GCS) or PGE₂ (1-4). Cellular activation must be achieved before the regulatory influences by these agents on the programming of T cell behavior are fully realized. Therefore, to appreciate the myriad of immunobiologic changes which accompany aging (8, 9), stress (10, 11), trauma (12-14), or even the maturation of immunologic responses in normal animals (15-17), requires that the experimental systems employed are not themselves altering cellular behavior in ways that might lead to erroneous conclusions.

Our research efforts have recently been focused on the nature, types, and biologic consequences that follow a T cell's encounter with natural endogenously produced steroid hormones (4, 5, 18). We have been able to demonstrate that many types of natural steroid hormones are able to alter T cell behavior in definable, hormone-specific manners by employing serum-free systems *in vitro* (18). We now report that the use of serum-supplemented medium restricts activated T cells to predominantly produce IL-2 after activation. The causative factor in serum was established to be platelet-derived growth factor (PDGF),¹ which is abundantly present in virtually all sources of serum. PDGF is not normally present in the lymphoid organ microenvironments where most T cells reside. Instead, PDGF represents a molecule that is primarily restricted in its action to paracrine pathways and exists in abundance at tissue sites of inflammation or injury where platelets are being activated. A number of the more important implications of our findings are discussed.

Materials and Methods

Mice

Male and female C57BL/6 strain mice were bred and housed in the Laboratory Animal Facility at the University of Utah from breeding stock originally purchased from the National Cancer In-

stitute. Mature adult mice of both sexes were used as LN donors and ranged in age from 12 to 20 wk. In any given experiment, pools of peripheral LN (axillary, brachial, and inguinal) from three to five mice (age and sex matched) were pooled. The animal facility at the University of Utah guarantees strict compliance with regulations established by the Animal Welfare Act.

Reagents

Antibodies. The mAb reagents used in the present study were obtained from a variety of sources. The anti-IL-2 (S4B6), anti-IL-4 (11B11), anti-IFN- γ (XMG1.2 and R46A2), and anti-IL-5 (TRFK4 and TRFK5)-producing hybridomas were obtained from either the American Type Culture Collection (Rockville, MD), or as gifts from Dr. T. Mossman and Dr. R. Coffman (DNAX, Palo Alto, CA). The cell line producing the hamster anti-murine CD3 ϵ mAb 145.2C was obtained from Dr. J. Bluestone (University of Chicago, Chicago, IL). Each of the cell lines was adapted to growth under serum-free conditions (see culture conditions) to facilitate antibody purification from cell supernatants. The anti-IL-4 mAbs (BVD4-1D11 and BVD6-2462) were obtained from PharMingen (San Diego, CA).

Bioactive Compounds. Murine rIL-2 and rIL-4 were obtained from Collaborative Biomedical Products, Inc. (Bedford, MA). Murine rIFN- γ was obtained from Genentech (San Francisco, CA), and murine rIL-5 was a gift from Dr. Robert Coffman (DNAX). These reagents were used as reference standards in the bioassays for IL-2, or in the capture ELISAs used to quantitate the other lymphokines. Human rPDGF-AA, PDGF-AB, and PDGF-BB were obtained from Boehringer Mannheim Corp., (Indianapolis, IN).

Culture Conditions

Routinely, single cell suspensions of LN cells were prepared from a group of normal mice, washed twice in sterile balanced salt solution, and resuspended at a density of 10⁷ cells/ml in a 24-well plate (Falcon Plastics, Baxter Scientific Products, Sunnyvale, CA). Culture medium consisted of RPMI 1640 supplemented with 1% Nutridoma-SR (Boehringer Mannheim Corp.) antibiotics, 2 mM L-glutamine, and 5 \times 10⁻⁵ M 2-ME. In some experiments, 10% FCS or PDGF-depleted FCS (Hyclone Laboratories, Logan, UT) was used in place of the Nutridoma supplement. Lymphocytes were activated by the addition of 1 μ g/ml anti-CD3 ϵ and supernatants collected after 24 h or when indicated. Cultures were incubated at 37°C in a 10% humidified incubator. Preliminary studies have indicated the cell density, incubation periods, and anti-CD3 ϵ concentration to be optimum for lymphokine production using Nutridoma-supplemented medium. Culture supernatants were harvested, clarified by centrifugation, and stored at 4°C until assayed for specific lymphokine content.

Preparation and Stimulation of T Cell Hybridomas

The method of Kappler et al. (19) was used to construct a panel of antigen-specific T cell hybridomas. All established clones have been recloned or retested for their capacity to produce IL-2, IL-4, and IL-5 after appropriate activation. Each of the hybridomas used in this study produce consistent patterns of these lymphokines when stimulated with soluble anti-CD3 ϵ plus accessory cells (e.g., LB15.13), or when cultured on 24-well plates containing immobilized anti-CD3 ϵ . Immobilized anti-CD3 ϵ plates were prepared by placing 250 μ l of a 1- μ g/ml solution of anti-CD3 ϵ in 0.01 M Tris-HCl (pH 9.6) into each well of a 24-well plate for 24 h at 4°C, followed by extensive washing with sterile balanced salt solution.

¹ Abbreviation used in this paper: PDGF, platelet-derived growth factor.

Lymphokine Assays

IL-2. The lymphokine-dependent cell line, HT-2, has been adapted to grow under serum-free conditions (RPMI 1640 + 1% Nutridoma SR) in the presence of 20 U/ml of rIL-2. Murine rIL-2 used for propagation of HT-2 was derived from culture supernatants of X6Ag8-653 cells (received from F. Melchers, Basel Institute Immunology, Basel, Switzerland) that had been transfected previously with multiple copies of the murine IL-2 gene (20). This cell line has also been adapted to growth under serum-free conditions. We have modified the bioassay of Hansen et al. (21) to utilize HT-2 as an indicator cell line to determine the relative titer of IL-2 produced by activated T cells. Each test supernatant was titrated in duplicate into serum-free medium against a constant number of HT-2 and a constant amount of anti-IL-4 (11B11). During the final 4 h of a 24-h incubation period, 5 μ g of 2-[4,5-dimethylthiazole-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) was added to each culture. After the 4-h period, 100 μ l of a 20% SDS/50% dimethylformamide solution was added to each culture well. Spectrophotometric readings at 570–650 nm were made on a V_{max} 96-well microtest plate spectrophotometer (Molecular Devices Corp., Menlo Park, CA). One unit of IL-2 in a test supernatant was equivalent to the optical density of a half-maximal response to the standard rIL-2.

Capture ELISAs for IL-4, IL-5, and IFN- γ . IL-4, IL-5, and IFN- γ levels in test supernatants were quantitatively measured by specific ELISA according to modifications of the method of Schumacher et al. (22). Briefly, a purified population of antilymphokine mAb (anti-IL-4, anti-IL-5, or anti-IFN- γ) was adsorbed to 96-well culture wells at a concentration of 2 μ g/ml in 0.05 M Tris-HCl (pH 9.6). Various amounts of lymphokine reference standards of known biologic activity, or dilutions of test supernatants, were dispensed into wells containing the specific capture antibody. After a 90-min incubation at 37°C, all wells were washed carefully with 0.05%

Tween-20 in PBS. The second antilymphokine antibody, which was biotinylated, was then added, incubated for 90 min at 37°C, and given another extensive washing in Tween-PBS. Avidin-horseradish peroxidase treatment, followed by the addition of ABTS-substrate, was used to develop the ELISA. Optimal density readings at 405 nm were taken using a V_{max} 96-well microtest plate spectrophotometer. Our limit of detection for IL-4 is 15–30 pg/ml, for IL-5 is 30 pg/ml, and for IFN- γ 30–60 pg/ml. The capture ELISA method has proven to correlate quite closely with standard bioassays for detection of the same lymphokines.

Results

Serum Effects on Lymphokine Production by Activated T Cells. Lymphocytes were collected from nonmucosal LN (axillary, brachial, and inguinal), mucosal LN (deep cervical, parathymic, and periaortic), and spleen of normal C57BL/6 strain mice. These three populations of lymphoid cells were then prepared for in vitro culture under serum-free and serum-containing conditions (10% FCS), and stimulated with an optimum amount of anti-CD3 ϵ . 24 h later, culture supernatants were collected and quantitatively analyzed for the presence of IL-2, IL-4, IL-5, and IFN- γ . The results presented in Fig. 1, A–D indicate that the patterns of lymphokine production are quite different between activated T cells stimulated under serum-free and those stimulated under serum-containing conditions. The presence of FCS augmented the amount of IL-2 produced by T cells from all three lymphoid compartments (Fig. 1 A), while simultaneously reducing the production of IL-4 (Fig. 1 B), IL-5 (Fig. 1 C), and IFN- γ (Fig. 1 D).

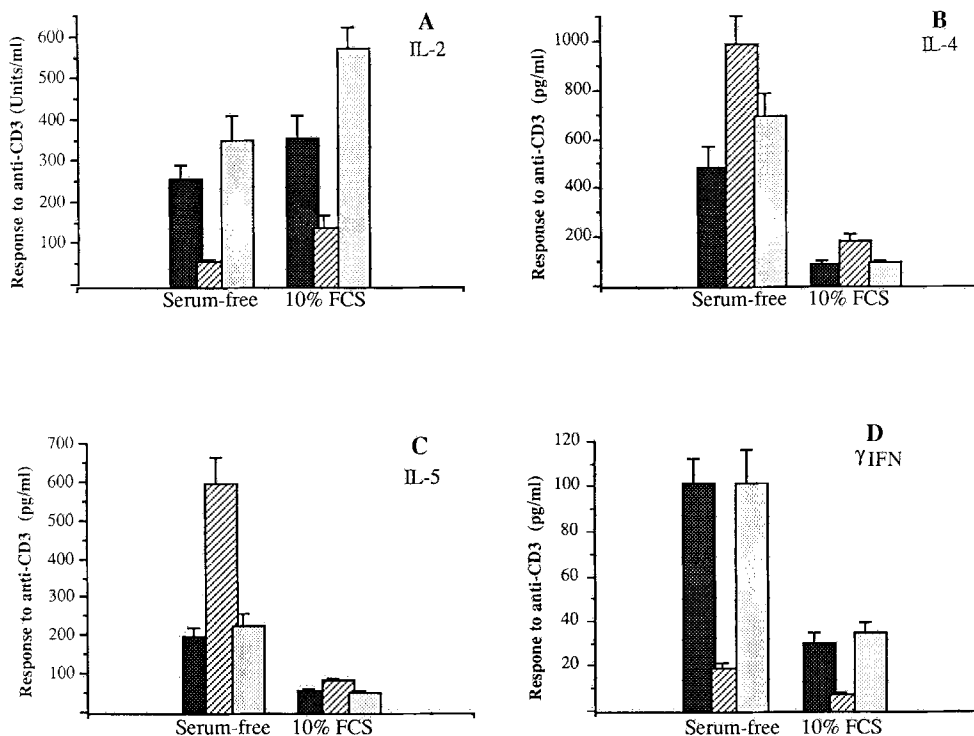


Figure 1. T cells activated in vitro produce different patterns of lymphokines when activated under serum-free or serum-containing conditions. Lymphocytes were obtained from nonmucosal LN (axillary, brachial, and inguinal) (■), mucosal LN (parathymic, periaortic, and deep cervical) (▨), and spleens (□) of normal C57BL/6 strain mice. 10^7 cells/ml were cultured in the absence (1% Nutridoma SR) or presence of 10% FCS with the addition of an optimum amount of soluble anti-CD3 ϵ (1.5 μ g/ml). Supernatants were collected after 24 h and quantitatively analyzed for the described lymphokines. Unstimulated control cultures, in the presence or absence of serum, were unable to produce detectable levels of the lymphokines tested. This experiment has been repeated four times with similar results.

To question whether the presence or absence of serum might also affect lymphokine production by homogenous T cell populations, we analyzed lymphokine synthesis by a number of T cell hybridomas. All of our T cell hybridomas have been adapted to grow under serum-free conditions (1% Nutridoma SR), thereby allowing us to maintain them without any possible serum-mediated influences. When activated in vitro with soluble anti-CD3 ϵ , in the presence of an accessory cell source (LB15.13), each T cell hybridoma produces a characteristic and reproducible pattern of the lymphokines IL-2 and IL-4. We found that T cell hybridomas, similar to freshly isolated lymphocytes, produced different amounts of IL-2 and IL-4 after activation under serum-free and serum-containing conditions. A representative example is provided in Fig. 2 that clearly demonstrates that the inclusion of 10% FCS in the culture medium enhanced IL-2 production while simultaneously inhibiting the production of IL-4. Identical changes in the pattern of IL-2 and IL-4 production are consistently observed with all of the T cell hybridomas tested ($n = 4$, data not shown). These experiments have been repeated numerous times with similar results. The very clear distinctions in the patterns of lymphokines produced by T cells residing within mucosal or nonmucosal lymphoid organs of normal mice when

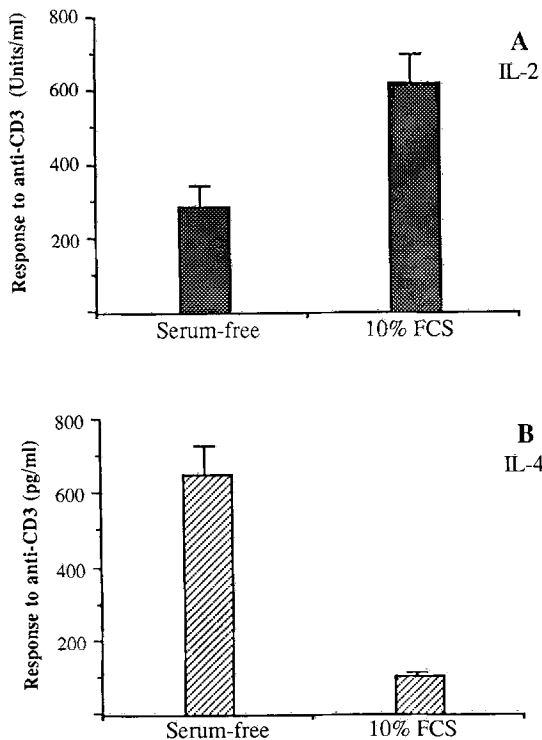


Figure 2. T cell hybridomas stimulated under serum-free or serum-containing conditions respond differently to stimulation with anti-CD3 ϵ . The T cell hybridoma HBD13.2 has been adapted to grow in serum-free medium (see Materials and Methods). Hybridoma cells (5×10^5 /ml) were cultured in the presence or absence of 10% FCS with 2×10^5 /ml LB15.13 as a source of accessory cells. Parallel sets of cultures were stimulated with 1 μ g/ml anti-CD3 ϵ and the supernatants collected and analyzed after 24 h. Both IL-2 and IL-4 productions were quantitated by the procedure described in Materials and Methods. Unstimulated T cell hybridomas in the presence of LB15.13 produce undetectable levels of the lymphokines tested.

stimulated under serum-free conditions is quite consistent with our previously published findings (23), while the patterns of lymphokines produced by T cells stimulated under serum-containing conditions (a predominance of IL-2 with minimal production of the other lymphokines) is similar to the published reports by other investigators (15–17, 24).

A significant emphasis has recently been placed on the value of quantifying the potential of T cells to produce lymphokines through an assessment of the patterns of these secreted products found in cell supernatants after T cell activation. Because of the very well-described activities of individual lymphokine species, quantitative and kinetic assessments of their production have been used as indicators of host immunocompetence (25–27), the effects of drug therapies (1, 3, 11), and the maturational status of the immune system of naive animals (15–17, 24, 28, 29). An accurate reflection of the in situ conditions is therefore quite important.

Serum contains a number of substances that are not generally part of a normal T cell microenvironment when residing within a lymphoid organ. Most notable are the many potent growth factors normally sequestered within blood platelets that are released as a consequence of clot formation. These include PDGF, transforming growth factor α (TGF- α), and transforming growth factor β (TGF- β). With the exception of TGF- β , which is known to be quite immunosuppressive (30, 31), the role(s) played by other platelet-derived substances on the behavior of T cells has not been characterized.

PDGF Regulates Lymphocyte Function. Based on the recent report that documented the existence of PDGF receptors on human T cells (32), we were led to question whether serum PDGF was responsible for the observed influences on T cell behavior in vitro. T-cell hybridoma cell lines were propagated under serum-free conditions and cultured in the presence of medium supplemented with 10% FCS, 10% PDGF-depleted FCS, 1% Nutridoma SR, or 1% Nutridoma SR plus 1 ng/ml PDGF-AB. All cultures were stimulated with an optimum amount of immobilized anti-CD3 ϵ and supernatants collected for lymphokine analysis 24 h later. The results of this experiment (Fig. 3) demonstrate that PDGF can significantly alter lymphokine production by activated T cells. Similar to the changes observed with serum, T cells placed into PDGF-AB-supplemented medium produced enhanced levels of IL-2 (Fig. 3 A), and were almost totally inhibited in their production of IL-4 and IL-5 (Fig. 3, B and C). Although some additional undefined factors may have also been removed, supplementation of the T cell hybridoma growth medium with PDGF-depleted FCS resulted in responses quite similar to those obtained under serum-free conditions. Therefore, PDGF appears to provide a major influence on T cell function, simultaneously enhancing the production of certain T cell lymphokines (IL-2) while profoundly inhibiting the production of others (e.g., IL-4 and IL-5).

PDGF Influences on T Cells Is Mediated through a β -type Subunit Receptor. PDGF is known to be encoded by separate genes for two possible subunit chains (A chain and B chain) which can form three possible dimers (AA, AB, and BB) (33–37). Competition studies have recently determined

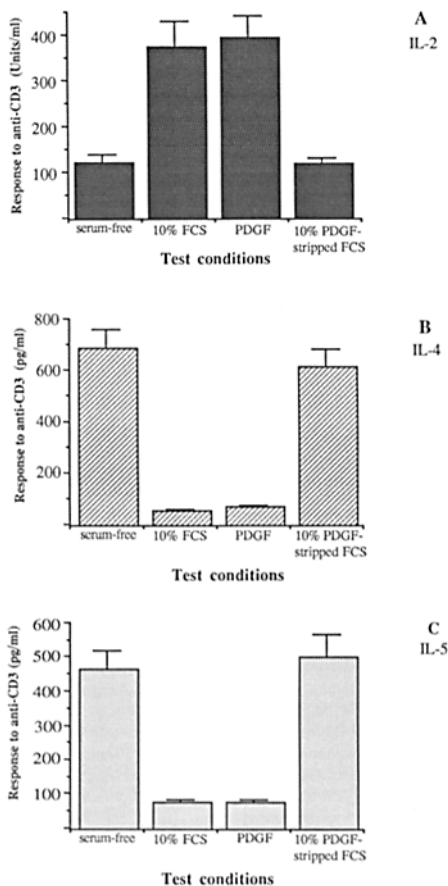


Figure 3. PDGF appears to be responsible for the restriction in lymphokine production by T cells cultured in serum-containing medium. The HBD13.2 T cell hybridoma was propagated in serum-free medium. Aliquots of these cells (5×10^5 /ml) were placed in medium containing the supplements described and immediately added to culture plates containing immobilized anti-CD3 ϵ . Supernatants were collected after 24 h and analyzed quantitatively for IL-2 (A), IL-4 (B), and IL-5 (C) (Methods). Control values were below the level of detection in our assays. This experiment has been repeated five times on many T cell hybridomas.

that different isoforms of PDGF bind to distinct classes of PDGF receptors (36). Present evidence indicates that high affinity binding of PDGF requires the association of two different receptor subunits: an α subunit that can efficiently bind to either A or B chains, and a β subunit that can bind only a B chain. Therefore, PDGF-AA is restricted to binding α/α receptors only, PDGF-AB binds to both α/α and α/β type receptors, while PDGF-BB can form high affinity complexes with either α/α , α/β , or β/β type receptors (33, 36).

We questioned whether PDGF-mediated alterations to T cell function were restricted to particular types of PDGF receptors. T cell hybridomas maintained in serum-free medium were stimulated with immobilized anti-CD3 ϵ in the presence or absence of various amounts of added PDGF-AA, PDGF-AB, or PDGF-BB. Results of the analysis on their ability to produce lymphokines (Fig. 4) demonstrated that PDGF-AA was ineffective, while PDGF-AB and PDGF-BB were highly effective at altering the production of IL-2, IL-4, and

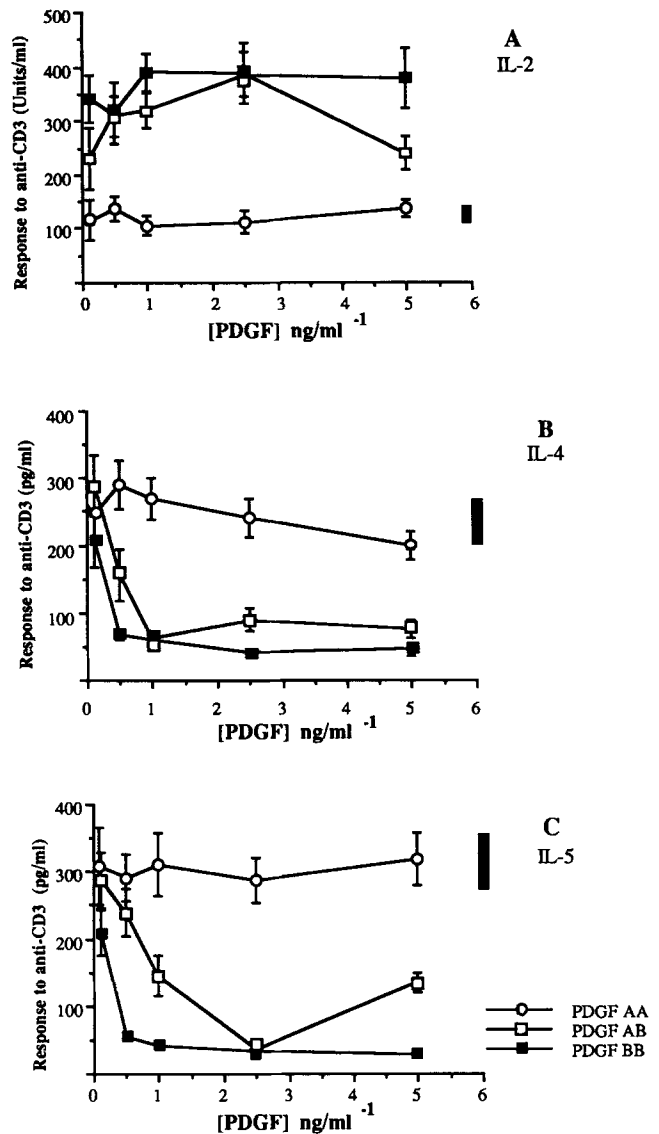


Figure 4. The PDGF influences on T cell function are dose dependent and appear to be mediated through a β -type receptor subunit. The T cell hybridoma T68.2 was placed into culture at 5×10^5 cells/well in the presence of the specified amounts of human rPDGF-BB, PDGF-AB, or PDGF-AA, and stimulated with immobilized anti-CD3 ϵ . Supernatants were collected after 24 h and assayed for the presence of IL-2 (A), IL-4 (B), and IL-5 (C). Control values were below detectable limits. This experiment has been repeated two additional times with different T cell hybridomas. The levels of each lymphokine produced by activated T cell hybridomas in the absence of PDGF \pm SEM are provided in the bars at the right of each figure.

IL-5 by activated T cells at most concentrations tested (0.1–5 ng/ml). The use of immobilized anti-CD3 ϵ eliminated the need for accessory cell addition to the culture system, allowing us to determine that the PDGF-mediated influences were directly related to T cell effects. Similar findings were obtained when freshly isolated lymphocytes from peripheral LN were used in a parallel analysis (Fig. 5). Finding that the production of IFN- γ by activated T cells was inhibitable by PDGF-BB at low concentrations, but by PDGF-AB only at

the highest concentration tested, was unexpected. Presently, we have no explanation for this finding, but it may relate to differential receptor subunit expression on T cell subsets. Our results suggest that both the stimulatory effects of PDGF (AB and BB) on IL-2 production and the inhibitory effects on IL-4, IL-5, and IFN- γ (BB only) production by the growth hormone appear to require a β subunit of the PDGF receptor being present on the responsive T cells.

PDGF Differentially Affects the Kinetics of Lymphokine Production by Activated T Cells. Freshly isolated peripheral LN lymphocytes were cultured in serum-free medium in the presence or absence of 10% FCS, 1 ng/ml of PDGF-BB, or 2.5 ng/ml of PDGF-AB. Cell cultures were stimulated by the addition of anti-CD3 ϵ , and supernatants collected at 24 and 48 h for quantitative lymphokine analysis. The results (Fig. 6, A-D) indicate that maximal levels of IL-2, IL-4, IL-5, and IFN- γ are achieved by 24 h when lymphocytes are activated under serum-free conditions, and these levels change minimally over the second 24-h period. T cells cultured in the presence of serum or PDGF (BB or AB) demonstrated similar kinetics for IL-2 production, with maximal levels achieved by 24 h. Conversely, very little IL-4, IL-5, and IFN- γ were produced by T cells activated in the presence of serum or PDGF-BB over the initial 24-h period. After 24 h in culture, however, significant IL-4, IL-5, and IFN- γ production were observed

over the second 24-h period in culture. Once again, PDGF-AB, while being stimulatory to IL-2 and inhibitory to IL-4 and IL-5, had no effect on the production of IFN- γ . Down-regulation of IFN- γ production therefore appeared to be restricted to PDGF-BB only. The results of this experiment suggest that cellular adaptation (or desensitization) to the effects of PDGF are taking place over the initial 24-h period, and further suggest that the regulatory effects by this growth hormone are transitory in nature.

Our findings suggested that PDGF-responsive T cells, placed into a PDGF-rich microenvironment (AB or BB), initially possess but then lose their sensitivity to this growth factor. We tested this directly by employing T cell hybridomas that had previously been adapted to in vitro growth under serum-free conditions. The results of this study are presented in Fig. 7. As previously demonstrated, T cell hybridomas maintained in serum-free medium were highly responsive to the effects of both PDGF-AB and PDGF-BB, as evidenced by an enhancement in IL-2 production and a marked depression in the levels of IL-4 and IL-5 when activated with immobilized anti-CD3 ϵ . Parallel cultures were maintained in the presence of 10 ng/ml PDGF-BB for a 48-h period. These cells were then washed free of growth hormone and activated with immobilized anti-CD3 ϵ in the presence or absence of freshly added PDGF-AB (2.5 ng/ml) or -BB (0.5 ng/ml). The results

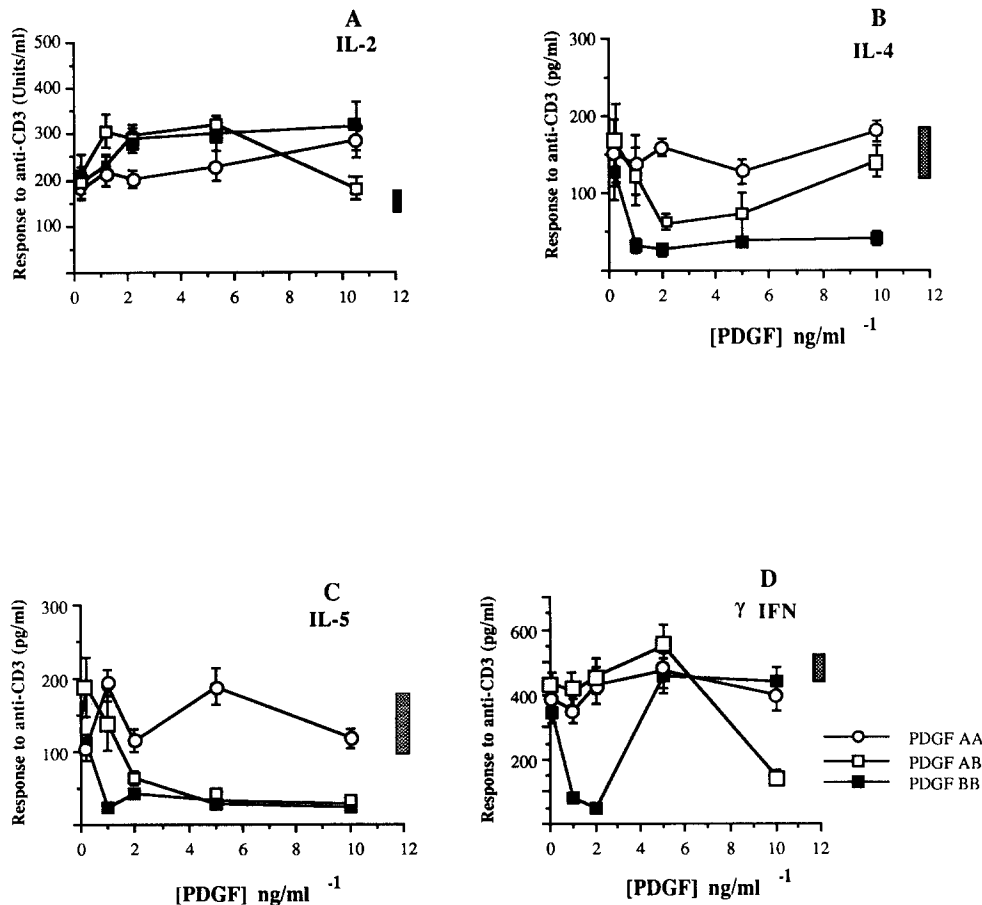


Figure 5. Dose-response effects of PDGF on murine LN T cells. Peripheral LN were harvested from C57BL/6 mice and prepared for culture in serum-free medium at a concentration of 10^7 cells/ml. 1-ml cultures were established in 24-well plates. Each of the isoforms of PDGF (AA, AB, BB) was added to duplicate wells at the indicated concentrations. The T cells were activated by the addition of 1 μ g/ml anti-CD3 ϵ . Culture supernatants were harvested after 24 h and quantitatively assayed for IL-2, IL-4, IL-5, and IFN- γ . Unstimulated, normal, and PDGF-only controls produced lymphokines below the level of detection in our assays. This experiment has been repeated twice. Positive control (minus PDGF) values for each lymphokine \pm SEM are provided in the bars to the right of each figure.

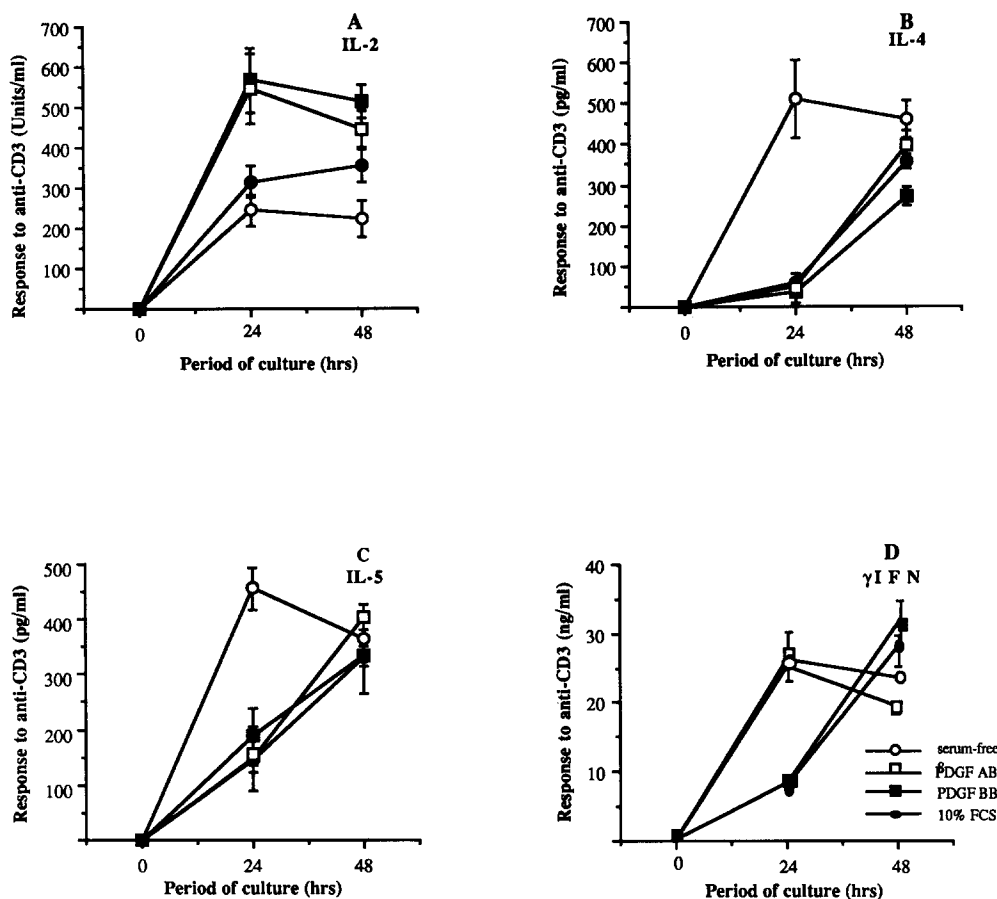


Figure 6. Kinetic analysis of lymphocyte production by murine peripheral LN cells in the presence or absence of PDGF-BB, -AB, or serum. Peripheral LN cells from C57BL/6 mice were prepared for culture at 10^7 cells/ml under serum-free conditions. To parallel culture wells, 2.5 ng/ml PDGF-AB, 1 ng/ml PDGF-BB, or 10% FCS were added just before activation with 1 μ g/ml anti-CD3 ϵ . Positive controls consisted of cells activated without added supplements. All cultures were activated by the addition of 1 μ g/ml anti-CD3 ϵ and supernatants from duplicate wells for each condition were harvested after 24 and 48 h. IL-2, IL-4, IL-5, and IFN- γ levels were quantitated as described in Materials and Methods. Unstimulated control values were below detectable limits and this experiment has been repeated twice with similar results.

demonstrated that PDGF-adapted cells became refractory to the regulatory influences of this growth factor as a consequence of being maintained in PDGF-containing medium. We have repeated this type of experiment many times, employing either PDGF or 10% FCS in the adaption culture system. The results were always identical to those presented above (data not shown).

Many cell types are rendered nonresponsive to the modulatory influences of specific polypeptide hormones by maintaining them in their physical presence. This process is termed "desensitization," "downregulation," or "adaption," and is often accompanied by a reduction in the number of membrane-associated hormone receptors. Cellular desensitization to PDGF is a well-described phenomenon in numerous cell types, is associated with a loss in surface receptor presence (38), and should be reversible when PDGF-desensitized cells are subsequently maintained under hormone-free conditions. We therefore questioned whether PDGF-desensitized T cell hybridomas could reacquire sensitivity to this growth factor. T cell hybridomas, desensitized to the influences of PDGF by a 48-h culture period in its presence (10 ng/ml of PDGF-BB), were subsequently incubated under PDGF-free conditions for a 24-h period. These cells were then activated by immobilized anti-CD3 ϵ in the presence or absence of added PDGF-AB or -BB. The results (Fig. 7, A-C) demonstrate

that desensitized T cells can reacquire sensitivity to both isoforms of PDGF after maintenance under PDGF-free conditions. Once, again, PDGF-AB and PDGF-BB were able to enhance the ability of these activated cells to produce IL-2, while simultaneously depressing their production of IL-4 and IL-5.

Discussion

Our findings indicate that serum, specifically the PDGF within serum, is able to exert significant influences on T cell behavior, which became evident subsequent to activation. T cells isolated from various lymphoid organ microenvironments, or T cell hybridomas, were found to respond quite differently in vitro, when stimulated with soluble anti-CD3 ϵ under serum-free or -containing conditions. T cells stimulated in the presence of serum were quite restricted in the types of lymphokines they produced, with IL-2 representing the predominant species. T cells stimulated under serum-free conditions, however, produced numerous species of lymphokines over the subsequent 24-h period, including IL-2, IL-4, IL-5, and IFN- γ . The primary factor in serum responsible for the observed restrictions in lymphokine production was determined to be PDGF, a potent peptide biological response modifier that previously has not been considered to be an

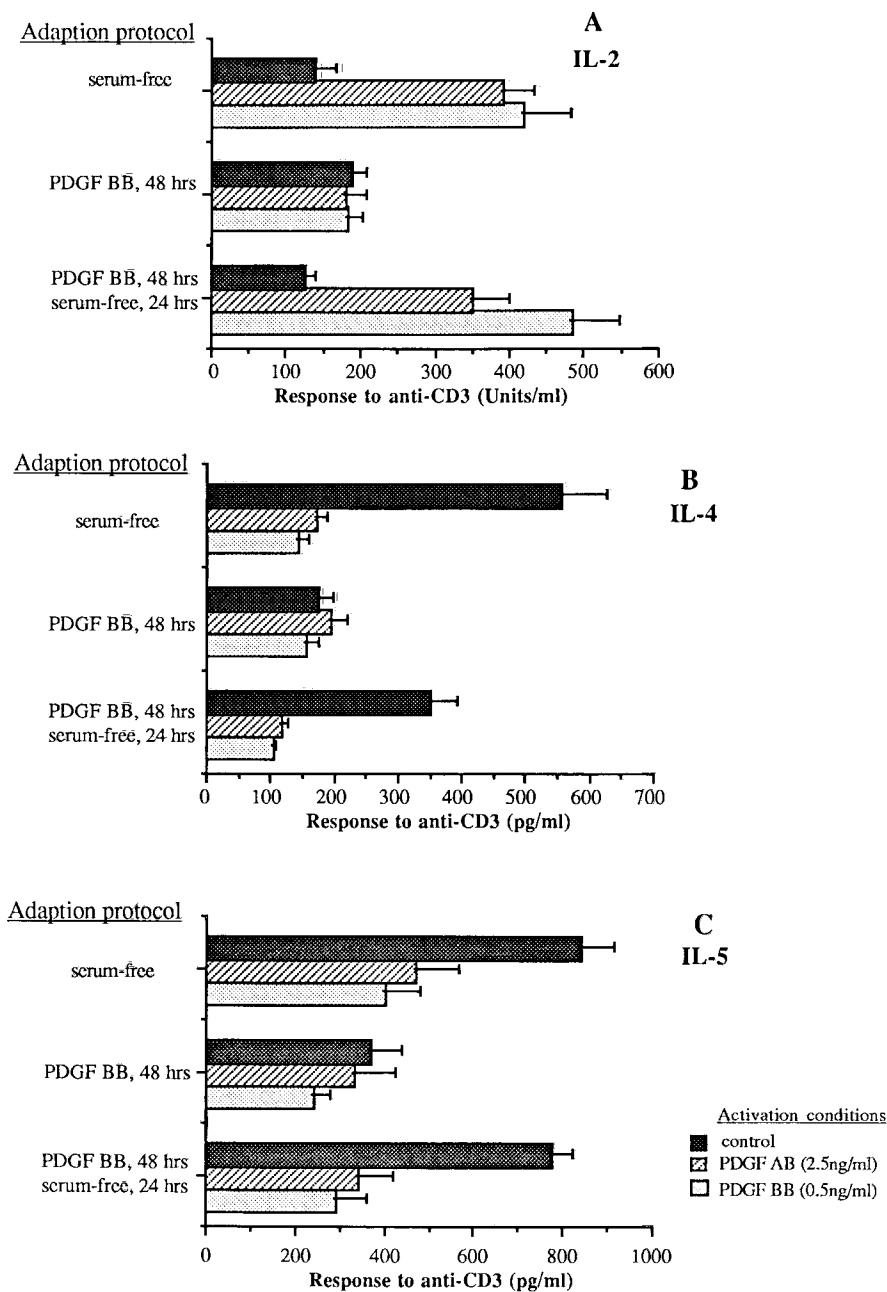


Figure 7. Lymphocytes cultured in PDGF lose sensitivity to this growth factor but regain responsiveness after its removal. The T cell hybridoma, Hd13.2, was maintained under serum-free conditions. A fraction of these cells were cultured with 10 ng/ml PDGF-BB for 48 h to achieve desensitization or adaption. At the end of the 48-h incubation period, both control and PDGF-treated cells were washed and resuspended at 5×10^5 cells/ml in serum-free medium. An additional set of cells were treated with PDGF-BB for 48 h followed by an additional 24-h period in its absence. The cells were activated on plates containing immobilized anti-CD3 ϵ in the presence or absence of PDGF-AB (2.5 ng/ml) or PDGF-BB (0.5 ng/ml). Supernatants were collected 24 h after activation and analyzed for levels of IL-2, IL-4, and IL-5.

important part of a T cell's microenvironment at time of activation.

PDGF actually represents a family of molecules that is composed of dimers of two chains (A and B chains). The two chains share 60% sequence identity and can dimerize to form PDGF-AA, PDGF-AB, and PDGF-BB. Each of the three dimer forms has been identified in nature and all are biologically active. The relative distribution of the three isoforms of PDGF varies greatly depending upon its cellular source of production, and probably relates to a variation in rate of A and B chain synthesis (39).

PDGF is able to stimulate a variety of cellular responses,

including mitogenesis, Ca^{2+} mobilization, actin reorganization, chemotaxis, phosphatidylinositol turnover, and stimulation of tyrosine-specific phosphorylation (reviewed in reference 33). Responses are elicited after PDGF binding to high affinity receptors, which themselves form plasma membrane dimers in the presence of ligand and gain biologic activity (36, 38). Two types of receptor subunits, termed α and β , are known to exist on individual cells. These receptor subunits can form homodimers or heterodimers, both of which are biologically active. The PDGF receptor-ligand system is further complicated by the findings that: (a) A chains can only interact with α receptor subunits, while B chains are fully

capable of high affinity binding to both α - and β -type subunits (36, 38); (b) the types of responses elicited through different α and β receptor isoforms may be distinct (33); and (c) variations in PDGF species and the relative proportion of either α or β subunits of the PDGF receptor in the plasma membrane can vary significantly depending upon the cell type plus secondary regulatory influences received by the cells (33, 40). Our findings (Fig. 4 and 5) strongly implicate the β subunit of the PDGF receptor as being necessary for altering the pattern of lymphokines produced by activated T cells. The ability of PDGF-AB and PDGF-BB to provoke the greatest responses suggests that most responding T cells must display both α and β subunits of the PDGF receptor, with the β subunit being necessary for modifying cellular behavior.

Two very obvious implications of our findings become immediately apparent. The first is associated with the almost universal usage of serum-supplemented culture medium in studies designed to elucidate the temporal sequence of molecular and biochemical events that follow T cell activation. Results derived from these types of studies have been interpreted by many investigators to reflect the immunologic competence of the lymphocyte donor under either normal or anomalous conditions (25–27). It is therefore very important to identify as many of the insidious factors present in a given experimental protocol that might also influence lymphocyte function and possibly contribute inaccurately to the interpretation of the data obtained. PDGF is a polypeptide hormone that contaminates all serum sources. In vivo, T cells would be most profoundly affected by PDGF influences when residing in areas of close proximity to sites of platelet aggregation and activation. Injury, inflammation, or infection, create unique *in vitro* microenvironments where transient PDGF-mediated effects on infiltrating lymphocytes might serve to promote the clonal expansion of specific antigen-responsive T cells.

It recently has been reported that the maturation of cellular responses *in vivo* begins with the activation of T cells that are predominantly restricted to the production of IL-2 when stimulated (15–17, 24, 28, 29). With time, the activated T cells are believed to “mature,” evidenced by the gaining of a capacity to produce a number of other lymphokines including IL-4, IL-5, and IFN- γ (6, 15, 17, 24). Our findings suggest that, at the population level, T cells *in vivo* possess the inherent potential to produce numerous lymphokines, but project the IL-2-dominant phenotype as a direct consequence of being programmed by PDGF-induced influences after their transfer from *in vivo* to an *in vitro* environment. The acquisition of an ability to produce a more diverse range of lymphokines, concluded by others to represent a normal consequence of lymphocyte maturation (6, 7, 24), may actually be the result of cellular adaption (or desensitization) to PDGF-mediated influences. The results of our experiments are consistent with this latter interpretation (Figs. 6 and 7).

It is very important clinically to accurately assess the immunologic status of individuals (either experimental animals or humans) whose immune systems are believed to be altered as a consequence of stress, trauma, drug therapies, aging, neo-

plasia, or challenge with particular infectious agents. Alterations in the patterns of lymphokines produced by T cells after their stimulation may serve as important indicators of a host's ability to mount protective or pathologic types of immunologic responses after antigenic challenge (11, 26, 27). An accurate assessment of the potential to produce the various species of lymphokines, both at the quantitative and qualitative levels, is necessary to derive the greatest usefulness from this information. We believe that our study indicates that the use of serum-supplemented medium in performing such evaluations places restrictions on the information that can be generated.

Serum contains numerous components that are able to exert profound influences over cellular behavior. These include not only PDGF, but all of the other biologic response modifiers that are normally physically restricted from generalized cellular contact by their incapsulation within platelets. In addition, the enzyme systems that are activated, and the numerous products produced as a consequence of blood clotting, also bear consideration. In hindsight, therefore, it is not at all surprising that T cell responses elicited under serum-free and serum-containing conditions would be quite different.

After appropriate types of stimulation, it is also possible for macrophages plus a number of other normal and neoplastic cell types to produce PDGF (41). The isoforms of PDGF produced by these cell types, either constitutively or in response to an exogenous stimulus, can vary in cell- and influence-specific manners (33). It is therefore possible that the PDGF effects on T cells are both dynamic and complex, and vary significantly within distinct tissue microenvironments.

We recently reported that exposure of T cells to glucocorticoids, either *in vivo* or *in vitro*, actually enhanced their capacity, upon stimulation, to produce IL-4 while depressing IL-2 and IFN- γ synthesis (4). This observation could not have been made in serum-containing medium, where the production of both IL-4 and IFN- γ is inhibited by PDGF effects. The use of a serum-free culture system has also allowed us to reproducibly observe differences in the patterns of lymphokines produced by T cells taken from distinct lymphoid organs (23). T cells resident within lymphoid organs that receive their predominant drainage from nonmucosal tissues produce significant amounts of IL-2 and IFN- γ following activation. T cells from mucosal lymphoid organs produce far more IL-4 and IL-5 after activation, and less IL-2 and IFN- γ than T cells from nonmucosal lymphoid organs. Our present findings offer an explanation as to why these differences were not previously observed in the past (Fig. 1). The potent depressive effects of PDGF on IL-4, IL-5, and IFN- γ production would minimize any inherent differences that may actually exist in the potential of T cells derived from the various lymphoid organs to respond to stimulation.

It is becoming quite clear that many substrates exist, with origins predominantly extrinsic to the T cell, that are able to greatly alter its cellular responses. These include the steroid hormones, with dehydroepiandrosterone (42), glucocorticoids (4), dihydrotestosterone (5), and 1,25-dihydroxy vitamin D₃ (18), each being capable of mediating distinct regulatory

effects on T cell behavior. The prostaglandins, via their ability to influence cyclic nucleotide levels in T cells, are also important regulators of T cell function (1). Our present finding that PDGF has profound influences over T cell behavior, coupled with its preexistence with blood platelets and its ability to be produced by numerous cell types, suggests an active participation by this growth hormone in the molecular mechanisms that govern the development of immunologic responses.

Lymphocytes and monocytes move freely between many distinct tissues as part of their roles in immune surveillance and effector function. Unlike cells that comprise the solid organ systems where microenvironmental influences are somewhat stable, the circulating mononuclear lymphoid cells are constantly being subjected to changing influences during the recirculation process. For optimal function, therefore, these cells must have the flexibility to rapidly adapt in an appropriate manner to a variety of distinct microenvironments. Fidelity in this process is necessary to guide the development and differentiation of appropriate effector responses after a lymphocyte's productive encounter with antigen within a particular tissue. PDGF may play more than one role in this process. By its apparent ability to limit lymphocyte production of lymphokines with differentiation functions while aug-

menting the production of IL-2, PDGF might amplify immune responses by its transient ability to focus the behavior of lymphocytes towards cellular proliferation. Clonal expansion is known to represent an absolute requisite for immunity and immunologic memory. A large dilution effect must occur where antigen-primed T cells enter the blood after residence, activation, and clonal expansion in a secondary lymphoid organ environment. For cellular immunity to occur, some of these antigen-specific T cells must selectively extravasate into inflammatory sites of infection and carry out their programmed functions. It is possible that PDGF influences, exerted locally, provide a means for additional clonal expansion of those T cells able to successfully traverse between the lymphoid organ and the site of infection.

Finally, it should be emphasized that this represents the very first report of a role for PDGF, beyond a suggestion for mitogenesis (32), in the regulation of functional roles played by T cells in the immune system. Finding that PDGF profoundly influences the functional behavior of T cells by altering their secretion of lymphokines, suggests an intimate linkage between this potent growth factor and the complex regulatory events associated with lymphocyte differentiation and the development of immunity.

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