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Effects of platelet-derived growth factor and epidermal growth factor on antigen-induced proliferation of human T-cell lines

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Summary. When the serum content of tissue culture medium is reduced from 10% to 1%, the capacity of T cells to proliferate in response to antigen within that medium is dramatically reduced. Physiological concentrations of platelet-derived growth factor (PDGF) or epidermal growth factor (EGF) are able to partially replace the requirement for serum, in that they are able to increase antigen-driven T-cell proliferation at a serum concentration of 1%. Neither growth factor is mitogenic for T cells in the absence of antigen, and neither is able to act synergistically with T-cell growth factor (TCGF) or IL-2) in the absence of antigen. Antigen-presenting cells (APC) pulsed with antigen in the presence of PDGF or EGF are able to stimulate antigen-specific T-cell proliferation to a greater extent than antigen-presenting cells pulsed in the absence of exogenous PDGF or EGF. Both growth factors increase the expression of MHC Class II antigens on antigen-presenting cells.

Abbreviations: AET, 5-2-aminoethylisothiourunium bromide hydrobromide; APC, antigen-presenting cells; E−, E-rosette negative; E+, E-rosette positive; EGF, epidermal growth factor; HAU, haemagglutinin units; HEPES, N′-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid; IL-2, interleukin-2; MHC, major histocompatibility complex; NP, nucleoprotein; PBL, peripheral blood mononuclear leucocyte; PDGF, platelet-derived growth factor; SDS, sodium dodecyl sulphate; TCGF, T-cell growth factor.

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INTRODUCTION

Most cell types require the presence of serum for DNA synthesis and cell division *in vitro*. In recent years, it has been shown that epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), both of which are present in serum, stimulate DNA synthesis and cell division in cells which otherwise require serum for the initiation of these processes (Carpenter, 1981; Westermark *et al.*, 1983; Deuel & Huang, 1983). These macromolecules interact with specific cell surface receptors (Carpenter, 1981; Westermark *et al.*, 1983; Deuel & Huang, 1983). Recent evidence has shown that the B chain of PDGF and a truncated form of the receptor for epidermal growth factor are related to the products of known oncogenes *sis* and *erb-B* (Doolittle *et al.*, 1983; Downward *et al.*, 1984; Johnsson *et al.*, 1984; Waterfield *et al.*, 1983; Ulrich *et al.*, 1984). These findings linking oncogenes to growth control are important for the understanding of the regulation of growth of normal, as well as of malignantly transformed, cells.

The growth factors EGF and PDGF act on a wide spectrum of cells. The EGF stimulates DNA synthesis in epidermal and epithelial cells (Carpenter, 1981) and PDGF has been shown to stimulate DNA synthesis in fibroblasts, smooth muscle and glial cells (Westermark *et al.*, 1983; Deuel & Huang, 1983; Scher *et al.*, 1979). PDGF can promote erythropoiesis *in vitro* (Dainiak *et al.*, 1983), as well as chemotaxis of monocytes, neutrophils, fibroblasts and smooth muscle cells (Westermark *et al.*, 1983; Deuel *et al.* 1982).

Although serum is required for antigen-specific immune responses *in vitro*, with few exceptions (Tees & Schreier, 1980; Needleman & Weiler, 1981; Tanno *et al.*, 1982), neither PDGF nor EGF have been investigated for a role in the regulation of antigen-driven division of lymphocytes. The experiments reported in this manuscript were designed to address this question. The results of these experiments show that both PDGF and EGF enhance antigen-driven T-cell proliferative responses at low serum concentrations, without being mitogenic in the absence of antigen. Further, the evidence obtained suggests that both growth factors act via regulation of antigen presentation and the expression of MHC Class II products, rather than directly on the proliferating T cells.

MATERIALS AND METHODS

Antigens

Formalin-inactivated influenza virus of the strain A/Texas/1/77 was obtained from Merck Sharpe and Dome Research Laboratories, Rahway, NJ. Influenza virus A/Brazil was the generous gift of Dr J. J. Skehel (National Institute of medical Research, Mill Hill, London). Purified nucleoprotein (NP; A/Texas/1/77) was generously provided by Dr K. Van Wyke, Laboratory of Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

Growth factors

Epidermal growth factor was obtained from Sigma Chemical Company (St Louis, MO). Porcine platelet-derived growth factor was the generous gift of Dr P. Stroobant (Imperial Cancer Research Fund, London) and was prepared as described elsewhere (Rozengurt *et al.*, 1983; Lopez-Rivas *et al.*, 1984). Both factors were purified to homogeneity (a single band on SDS polyacrylamide gel electrophoresis). Neither growth factor preparation contained detectable endotoxin, as assessed by the E-toxate test (Sigma Chemical Company).

Production of T-cell growth factor (TCGF)

TCGF used for the maintenance of the anti-NP T-cell line was prepared as described elsewhere (Lamb *et al.*, 1982a). Serum and lectin-free TCGF was prepared using the gibbon T-cell line MA 144: T cells ($1{\cdot}5 \times 10^6$/ml) were suspended in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 50 IU/ml penicillin, 50 μg/ml Streptomycin and 25 mM HEPES, and cultured for 3 days at 37°, 5% CO_2. The supernatant was harvested by centrifugation and passed through a 0·22 μm filter. Activity was assessed using a TCGF-dependent T-cell line, and was consistently equal to or greater than TCGF prepared from human tonsil cells and PHA.

Production and maintenance of a human T-cell line specific for influenza A virus nucleoprotein

A T-cell line reactive with influenza A virus NP was isolated as previously described. Briefly, lymphoblastoid cells from a 6-day culture of normal PBLs ($2{\cdot}5 \times 10^5$/ml) stimulated with NP (1·0 μg/ml) were isolated on a discontinuous Percoll gradient and cultured in the presence of autologous irradiated (3000 rads) PBLs (5×10^5/ml), NP (1·0 μg/ml) and TCGF. The T cells were expanded with fresh TCGF every 3–4 days, and irradiated histocompatible PBLs and A/Texas or A/Brazil virus added every 7 days. Prior to their use in proliferative assays, the line was rested for 7 days after the addition of filler cells.

Antigen-presenting cells

Unless otherwise indicated, antigen-presenting cells (APC) were peripheral blood mononuclear leucocytes (PBL), DR identical with the anti-NP T-cell line, which had been prepared and cryopreserved as described elsewhere (Lamb *et al.*, 1982b). PBL were thawed and irradiated with 3000 rads from a ^{60}Co source immediately prior to use. E-rosette negative cells were prepared from the PBL treated as described above by rosette formation with 5-2-aminoethylisothiouronium bromide hydrobromide (AET; Calbiochem, La Jolla, CA)-treated sheep erythrocytes. The non-rosette forming cells (E-) fraction ($< 1\%$ E+) was recovered after centrifugation over Percoll (1·080 g/ml; Pharmacia, Uppsala, Sweden). E+ Cells were recovered from the pellet by lysis of erythrocytes using Gey's haemolytic solution.

Proliferative assay

T cells from the anti-NP line were cultured with either A/Texas/1/77 (5HAU/ml) or A/Brazil (5HAU/ml) in the presence of irradiated APC, in RPMI 1640 (Gibco) supplemented as described above, in 96-well, round-bottomed plates (Flow Laboratories, Irvine, Scotland). After 40 hr in culture, cells were pulsed with 1 μCi tritiated thymidine ([^{3}H]TdR, Amersham, Bucks, U.K.), incubated for a further 8 hr and harvested onto

glass fibre paper (Watman, Maidstone, Kent). [^{3}H]TdR incorporation was measured using liquid scintillation spectroscopy. Antigen-specific proliferation by the NP line peaked sharply on day 2 of culture (Table 1).

Cell sorter analysis

Indirect immunofluorescence labelling was accomplished as described elsewhere (Lamb *et al.*, 1983b). Fluorescence intensity was measured on a logarithmic scale using a FACS IV. Monoclonal antibodies used were UCHT1 and Leu 3a, which have specificity for the T3 and T4 antigens, respectively; anti-Tac, which is specific for the IL-2 receptor; DA2, which is specific for a non-polymorphic determinant of HLA-DR; and 2A1, which is against a non-polymorphic MHC Class I determinant.

RESULTS

Ability of PDGF and EGF to enhance antigen-specific T-cell proliferation

The antigen-induced proliferative response of an IL-2 dependent T-cell line specific for NP is reduced in magnitude when the serum concentration of the culture medium is reduced below 3% (Fig 1a). At serum concentrations of 10% and 3% the addition of PDGF over a 3 log range of concentrations (0·01–1·0 units/ml) had no significant effect on antigen-induced proliferation (Fig. 1a). In contrast, antigen-specific proliferation at 1% serum is markedly enhanced by the addition of PDGF (Fig. 1a), but background [^{3}H]TdR incorporation, in the absence of antigen, remained unchanged. The optimum concentration was consistently 0·01–0·1 units/ml equivalent to 0·14–1·4 ng/ml. Thus, the enhancing effect of PDGF on T-cell proliferation is observed only in the presence of antigen and appears to have no mitogenic effect in the absence of antigen. Similar results are observed when EGF is added to antigen-induced T-cell proliferation in the presence of a limiting concentration of serum (Fig. 1b), in that EGF (0·01–0·1 ng/ml) enhansed T-cell proliferation at 1% serum, but does not affect background responses.

Table 1. Kinetics of the NP-specific line used (c.p.m. ± SE)

Days after initiation of culture	0 Ag background	+Ag	+Ag +0·1 ng/ml EGF
1	139 ± 52	1096 ± 132	1698 ± 158
2	1208 ± 343	5634 ± 363	10,438 ± 743
3	656 ± 72	1855 ± 594	ND*
4	450 ± 95	1196 ± 143	1157 ± 417

The observation that antigen-specific proliferation peaks sharply at day 2 and falls off dramatically at day 3 has been consistent in repeat experiments.

* ND, not done.

Effect of PDGF and EGF on IL-2 mediated proliferation

Since the experiments reported above do not discriminate whether PDGF and/or EGF exert their effects on antigen-presenting cells or on the T cells themselves, the effect of PDGF or EGF on IL-2 (TCGF) mediated proliferation was determined. IL-2 stimulates T-cell proliferation, independent of antigen-presenting cells (Gillis *et al.*, 1978). The addition of PDGF or EGF to the T-cell line stimulated with IL-2 at various concentrations (0·1–30%) did not increase T-cell proliferation markedly (Fig. 2a, b). As would be predicted, both PDGF and EGF increased T-cell proliferation in the presence of antigen, in the absence of and at low concentrations of IL-2 (Fig. 2a, b), since the cultures also contained antigen-presenting cells.

Analysis of growth factor activity on the function of the antigen-presenting cell population

In order to determine whether PDGF and/or EGF exert their effects on antigen-specific T-cell proliferation via T cells or via antigen-presenting cells, E-rosette negative (E−) cells were incubated with antigen alone, or with antigen and growth factors. Control E−cells were incubated without antigen, or without antigen but with PDGF or EGF. After 48 hr, the cells were washed and incubated with T cells of the NP-specific line, in the absence of any additional antigen or growth factors. The number of E− cells per culture is limiting, as shown by previous experiments (Lamb *et al.*, 1983a). Antigen pulsed E− cells caused T cells to proliferate, but E− cells pulsed with antigen in the presence of PDGF or EGF resulted in a marked increase in T-cell proliferation (Fig. 3). E− cells incubated without antigen, used as controls, caused minimal, background stimulation.

Phenotypic analysis of T cells and antigen-presenting cells following pretreatment with growth factors

Since antigen presentation involves the expression of MHC Class II molecules, we investigated whether

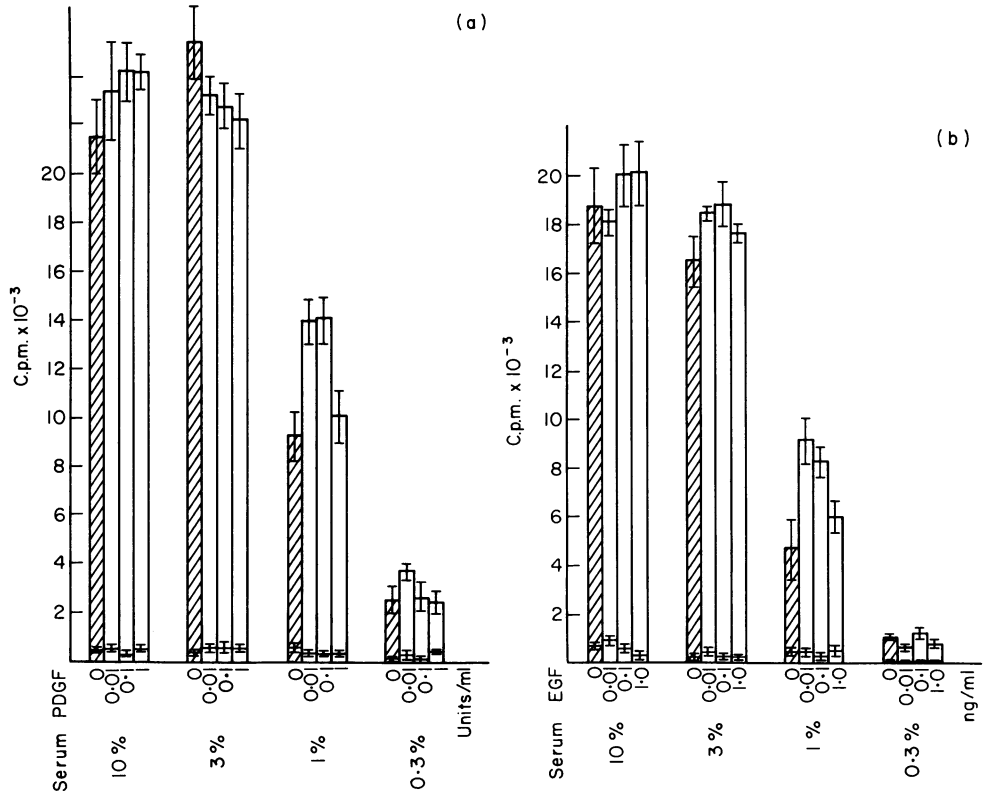

Figure 1. (a) The ability of PDGF to partially restore antigen-driven T-cell proliferation limited by low serum concentration. 10^4 cells from a continuously maintained, NP-specific line were cultured for 48 hr with $2{\cdot}5 \times 10^4$, irradiated, DR-matched PBL and A/Brazil influenza virus at 5 HAU/ml and various concentrations of human A+ serum, with no PDGF added (■) or with PDGF added at the concentrations shown (□) (1 unit/ml = 14 ng/ml). Cultures were pulsed at 40 hr with 1 μCi [^{3}H]TdR and harvested at 48 hr. Counts per minute (c.p.m.) shown represent the mean of triplicate or quadruplicate cultures ± 1 s.e. The short histograms (< 500 c.p.m.) indicate c.p.m. of control cultures incubated under the same conditions, but without antigen. (b) The ability of EGF to partially restore antigen-driven T-cell proliferation limited by low serum concentration. Details as in (a), substituting EGF for PDGF.

pretreatment of E− cells with PDGF or EGF resulted in increased expression of MHC Class II antigens, as determined by indirect immunofluorescence staining (Fig. 4). The increase in MHC Class II expression was very similar to that resulting from treatment with gamma interferon (Fig. 4). No effect on the expression of MHC Class I antigen was observed (not shown).

In contrast to their effect on the E− cell fraction, neither PDGF nor EGF had any discernable effect on MHC Class II expression of T cells. Furthermore, the expression of the IL-2 receptor, T3 and T4 antigens remained unaltered by these growth factors (Table 2).

DISCUSSION

We report here the effect of the growth factors PDGF and EGF on antigen-induced proliferation of human T lymphocytes. It was observed that the addition of picogram quantities of PDGF or EGF partially restores the antigen specific T-cell proliferative response which had been limited by the reduction of the serum content of the tissue culture medium.

From the known concentrations of PDGF in human serum (Singh, Chaikin & Stiles, 1982; Huang, Huang & Deuel, 1983), it can be estimated that

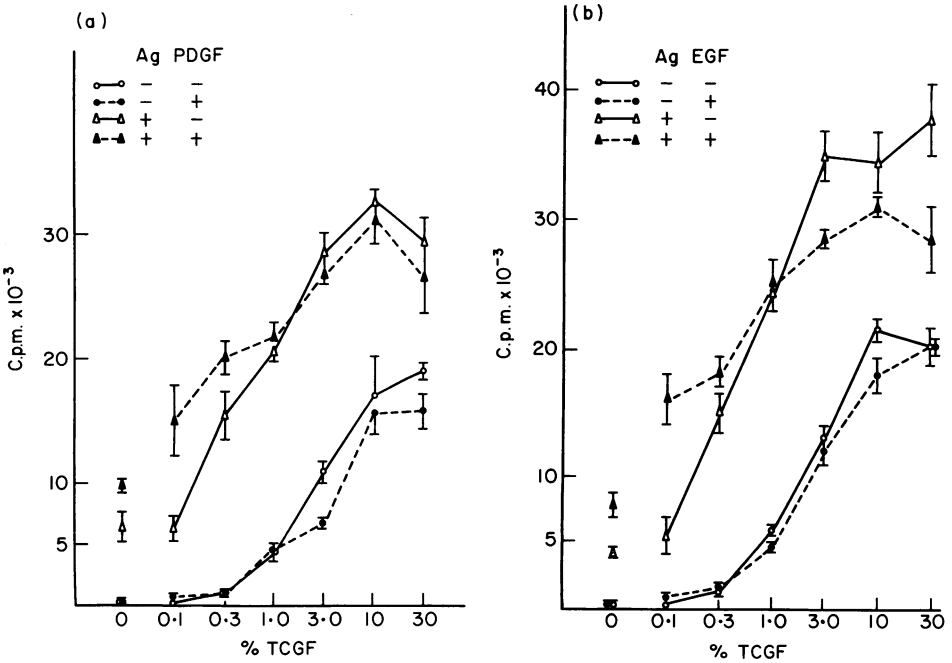

Figure 2. Effect of PDGF on TCGF-driven T-cell proliferation. 10^4 NP-specific T cells were cultured with $2{\cdot}5 \times 10^4$ APC and various concentrations of serum and lectin-free supernatant, known to contain TCGF, at a serum (A+) concentration of 1%. PDGF (●,▲) was added at a final concentration of 0·1 units/ml. Influenza virus A/Brazil (▲,△) was added at a final concentration of (5HAU/ml). Cultures were pulsed at 40 hr with 1 μCi [^{3}H]TdR and harvested at 48 hr. C.p.m. shown represent the means of triplicate or quadruplicate cultures ± 1 SE. (b) Effect of EGF on TCGF-driven T-cell proliferation. Details as in (a), substituting EGF for PDGF.

medium containing 1% serum would contain between 0·15 and 0·5 ng/ml PDGF. We found that the addition of 0·14–1·4 ng/ml purified porcine PDGF had maximal effects on antigen-driven T-cell proliferation. Porcine PDGF is comparable in biological activity to human PDGF (Lopez-Rivas *et al.*, 1984). Similarly, the EGF content of medium containing 1% serum can be estimated at 2·7–10 pg/ml (Oka & Orth, 1983; Carpenter, 1981). We found that the addition of 0·01–1·0 ng/ml EGF had maximal effects. These effective ranges of concentrations of PDGF and EGF are well within those that would be predicted, based on the assumption that decreasing serum content restricts antigen-driven T-cell proliferation by decreasing the concentrations of PDGF and EGF in the tissue culture medium. The findings suggested that PDGF and EGF were capable of influencing immune regulation. However, they did not elucidate the mode of action of PDGF and EGF.

In order to define the mode of action of PDGF and EGF on the T-cell proliferative response, which involves two cell types, T cells and antigen-presenting cells, we assayed EGF and PDGF on the response of T cells to IL-2, which does not require antigen-presenting cells. No effect was noted (Fig. 2). Since many ligands which influence T cells, e.g. phorbol esters (I. Ando, G. Hariri, D. Wallace and P. C. L. Beverley, submitted for publication) and antigen (Reinherz *et al.*, 1982; Zanders *et al.*, 1983), modulate T-cell surface antigen expression, we investigated whether the T3, T4 antigen or the IL-2 receptor expression on T cells was altered. No change was observed. These results suggested that the mode of action of EGF and PDGF was by an effect on the antigen-presenting cell function, which was confirmed by preincubation of E− cells with EGF or PDGF (Fig. 3). Antigen-presenting function is critically dependent on the expression of MHC Class II antigens, and so the effect of EGF and PDGF on the expression of MHC Class II antigens was ascertained in E− cells. The shift in fluorescence intensity from peak channels 135 to 164 or 172 can be approximated to correspond to a two- to three-fold

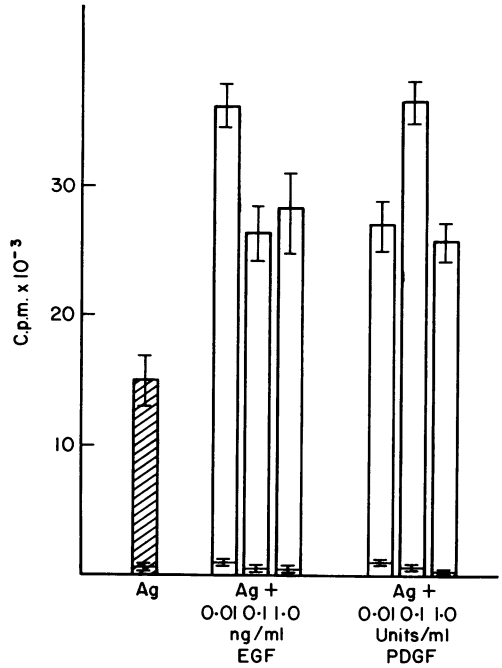

Figure 3. Effect of PDGF or EGF on antigen presentation by E-rosette negative (E−) cells. APC were prepared as described and incubated at 37° for 48 hr in medium containing 1% A+ serum with antigen (influenza virus A/Brazil) alone (■) or together with various concentrations of PDGF or EGF (□). After 48 hr, APC were washed three tinmes by centrifugation in medium containing 1% A+serum, and resuspended at a 1:1 ratio with RNP-specific T cells. No significant differences in viability between groups were observed. After a further 40 hr, cultures were pulsed with [^{3}H]TdR and harvested at 48 hr. C.p.m. represent the means of triplicate or quadruplicate cultures ± 1 SE. The short bars represent counts incorporated by cultures of T cells with control E − ve cells having a prior incubation with no antigen, or no antigen and various concentrations of PDGF or EGF.

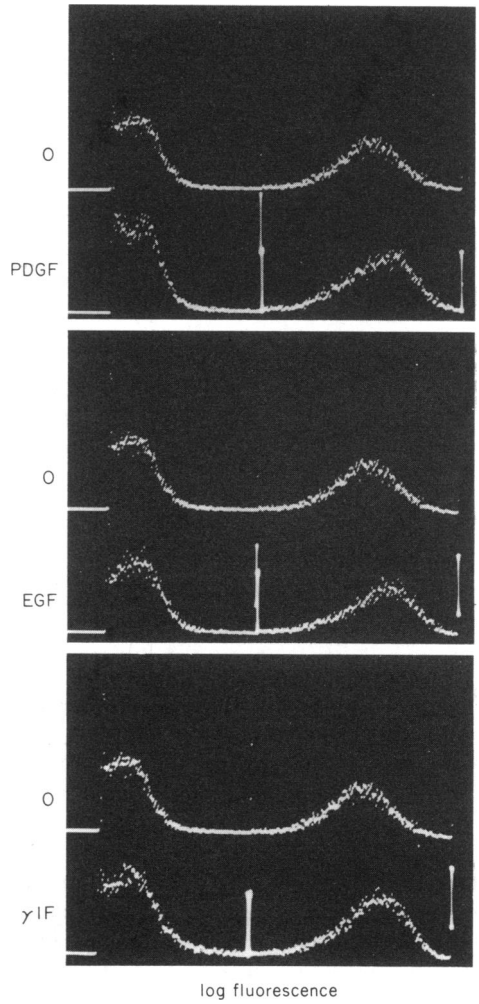

Figure 4. Shift in expression of MHC II antigen expression on APC as a result of exposure to PDGF, EGF or interferon. 10^6E− PBL were cultured in 1 ml medium containing 1% A+ serum alone, or supplemented with 0·1 units/ml PDGF, 0·1 ng/ml EGF or 1 IU/ml gamma interferon. After 96 hr in culture, cells were stained and analysed for fluorescence as described. Fluorescence intensity is displayed on a log scale. Peak fluorescence of control cultures was in channel 135; that in PDGF culture in channel 164, that in the EGF-treated culture 172, and that in the interferon-treated culture 162. Percent positive cells remained unchanged at 84%, 83%, 83% and 81% for control, interferon-, EGF- and PDGF-treated cells, respectively.

increase in fluorescence intensity, since a scale representing $\log_{3\cdot5}$ increases in fluorescence intensity is covered by channels 1–255. This is a population of B cells and monocytes, and it is not known whether the two-fold increase in MHC Class II detected by FACS IV involved one or both cell types, although both cell types are capable of antigen presentation (Chestnut & Grey, 1981). This question is currently under investigation. It is interesting that, while EGF, PDGF and gamma interferon all increase MCH II expression on peripheral blood mononuclear cells, gamma interferon does not appear to increase antigen-specific T-cell proliferation (data not shown) and, in some systems, has been shown to be inhibitory (Kadish *et al.*, 1980).

The finding that growth factors with effects on a wide spectrum of cells, usually at concentrations

Table 2. Growth factor-induced modulation of cell surface antigens as assessed by FACS analysis

Growth factor	Incubation time		Ag (Ab)	Modulation
EGF	24 hr	96 hr	T3 (UCHT1)	–
PDGF	"	"	" "	–
EGF	"	"	T4 (Leu 3a)	–
PDGF	"	"	" "	–
EGF	"	"	IL-2 rptr (Tac)	–
PDGF	"	"	" "	–
EGF	"	"	MHC II (DA2)	–
PDGF	"	"	" "	–

higher than those reported here, (Carpenter, 1981; Westermark *et al.*, 1983; Deuel & Huang, 1983) also influence the immune response, by an action on antigen-presenting cell function, has certain physiological implications. During wound or tissue repair, these growth factors would be liberated (Westermark *et al.*, 1983; Deuel & Huang, 1983; Oka & Orth, 1983), and their effects on augmenting MHC Class II expression and antigen presentation may assist in protecting against infection. These results also reinforce the evidence that regulation of HLA-DR repression and antigen presentation is of major importance in immune regulation (Bottazo *et al.*, 1983; Janeway *et al.*, 1984) and indicate that ligands other than interferon may modulate HLA-DR. Serum-free media for *in vitro* murine (Tees & Schreier, 1980) and human (Needleman & Weiler, 1981; Tanno *et al.*, 1982) immune responses, as assessed by mitogen-driven proliferation or antigen-specific antibody production, have been reported. From the findings reported in this manuscript, it is predictable that growth factors such as EGF and PDGF would be effective in helping to support an antigen-driven, human T-cell proliferative response under serum-free conditions. Alternatively, some serum components may be involved in the effects exerted by EGF and PDGF, or whose negative effects are overcome by EGF and PDGF.

Preliminary experiments suggest that EGF and PDGF are not significantly additive or synergistic in their immune modulating effects. Experiments to explore synergy between these and other growth factors in *in vitro* immune responses are currently under investigation in this laboratory.

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