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Regulation of human tonsillar T-cell proliferation by the active metabolite of vitamin D_3

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SUMMARY

We have examined the effects of 1,25 $(OH)_2D_3$ on T-cell populations isolated by buoyant density and E rosetting from human tonsils. Cell proliferation was assessed by measuring the incorporation of ^{125}iododeoxyuridine; interleukin-2 (IL-2) production was measured using an IL-2-dependent cell line, and the number of 1,25 $(OH)_2D_3$ receptors was measured by whole-cell nuclear association assay. At a concentration of 10^{-7} M, $1,25(OH)_2D_3$ inhibited mitogen-induced T-cell proliferation in all E^+ T-cell populations. This effect was more pronounced in the cells from the intermediate and high density layers and was reflected both in cell proliferative responses and in relative IL-2 synthesis. By adding the $1,25(OH)_2D_3$ during the course of the mitogen assay, we demonstrated that activation of the T cell precedes the $1,25(OH)_2D_3$-mediated inhibition. Cells that had been preincubated with mitogen in the presence of the $1,25(OH)_2D_3$ were refractory to further stimulation by mitogens. Receptors for $1,25(OH)_2D_3$ could not be detected in unstimulated T cells. However, activation led to the expression of high-affinity receptors for $1,25(OH)_2D_3$. Co-incubation of the cells with mitogen and $1,25(OH)_2D_3$ increased the number of receptors compared with mitogen alone. The effects provide further evidence for the hypothesis that $1,25(OH)_2D_3$ is an important potential modulator of the immune system through its action on T cells. Taking our observations in conjunction with the known capacity of monocytes to hydroxylate the precursor metabolite (and thus synthesize the active form of cholecalciferol), the results support the suggestion that $1,25(OH)_2D_3$ plays a role as a local mediator of mononuclear phagocyte–T cell interaction in human lymphomedullary tissues.

INTRODUCTION

There are several recent reports that implicate the active metabolite of vitamin D_3, namely 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$, also known as calcitriol) as an important mediator of the immune response both in murine experimental systems and in man.

One line of evidence is based upon the observation that $1,25(OH)_2D_3$ receptors are present both on activated normal human peripheral blood lymphocytes and on malignant lymphoid cells (Provvedini *et al.*, 1983). The metabolite acts as an inhibitor of both interleukin-2 (IL-2) synthesis and proliferation of these cells. Another possible role for $1,25(OH)_2D_3$ is in promoting cell differentiation in the mononuclear phagocyte system (Reitsma *et al.*, 1983). Also, $1,25(OH)_2D_3$ may act in stimulating resistance to mycobacterial disease via the mononuclear phagocyte regulatory pathway, thus suggesting an important biological role for the hormone in the pathogenesis of granulomas (Rook *et al.*, 1986).

In this study, we describe investigations that evaluate how $1,25(OH)_2D_3$ influences the response of human tonsillar T cells. Our results suggest that $1,25(OH)_2D_3$ may have a role in homeostasis within this T-cell pool as well as in the circulating population. This function may represent a T-cell autoregulatory mechanism that generates cells that are refractory to further response amplification.

MATERIALS AND METHODS

Cell separation

The starting population for the isolation of human tissue T cells consisted of a collagenase digest from human tonsil. Approximately 100 operative specimens were used in the different stages of these experiments. The tissue digest was prepared by

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Abbreviations: $1,25(OH)_2D_3$, 1,25-dihydroxycholecalciferol; E^+ cells, cells expressing receptors for sheep red blood cells; HD, high-density cells; ID, intermediate-density cells; IL-2, interleukin-2; ^{125}IUdR, 5-[^{125}I]iododeoxyuridine; LD, low-density cells; PHA, phytohaemagglutinin.

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incubating the tissue fragments in Collagenase II (Sigma, Poole, Dorset) at a final concentration of 1 mg/ml in calcium- and magnesium-free Hanks' solution for 1 hr at 37°. This was followed by centrifugation on a discontinuous bovine serum albumin gradient modified from the methods used previously to examine human (Geha & Merler, 1974) and murine (Sunshine, Katz & Feldmann, 1980) tissue populations. This procedure resulted in three different cell populations separable by density, termed low density (separating at less than 20% albumin concentration) (LD), intermediate density (separating between 23% and 26% albumin concentration) (ID), and high density (separating between 26% and 35% albumin concentration) (HD), respectively.

In order to purify T cells from these three subpopulations, the cells were first allowed to adhere overnight to a plastic substrate, followed by a standard rosetting technique for identification of cells expressing sheep red blood cell (E) receptors. The T-cell nature of these E^+ cells was confirmed by a rosetting method using UCHT1 (a CD3 anti-T cell monoclonal antibody, gift of Dr P. Beverley, Human Tumour Immunology Group, ICRF, London) and chromic chloride coupled complexes of ox red blood cell/rabbit anti-mouse immunoglobulin pre-absorbed against human immunoglobulin (Dako Ltd, Weybridge, Surrey) (Parrish & McKenzie, 1978). Controls included both irrelevant monoclonal antibodies (e.g. 3Ac5, gift of Dr J. Ledbetter, Seattle, WA) and antibodies against class II major histocompatibility complex determinants (e.g. clone DA6.231, gift of Drs Guy and van Huyningen, University of Edinburgh).

Cell proliferation

Cell proliferation assays were performed for different time-periods as required in the individual experiment (see below). After the initial cell separation, each cell population was resuspended in RPMI (Gibco, Paisley, Renfrewshire) supplemented with 5% heat-inactivated fetal calf serum, 100 IU/ml penicillin, 0·100 mg/ml streptomycin, 2·5 mg/ml amphotericin, 10 mM HEPES, 0·05 mM 2-mercapto-ethanol and 2 mM L-glutamine. Cultures were performed in triplicate in 0·2-ml volumes in a humidified 5% CO_2 atmosphere and at 37°. Phytohaemagglutinin (PHA) was used as a mitogen in the majority of the experiments; similar results have been obtained using pokeweed mitogen (data not shown). Six hours before harvesting, 5-[^{125}I]iododeoxyuridine (^{125}IUdR) (5 Ci/mg, Amersham International, Amersham, Bucks) was added to the cultures and the subsequent incorporation of the radiolabel into macromolecular DNA measured. Results were calculated as the mean c.p.m. $\times 10^{-3}$ of each triplicate, with standard deviation in these experiments as less than 20%.

In experiments where larger cell numbers were required, 5×10^6 cells were cultured in 10-ml volumes in 50 ml capacity tissue culture flasks (Nunc, Roskilde, Denmark) with or without mitogens and with or without the metabolite. The cells were harvested and either re-cultured or examined as necessary.

Vitamin D_3 metabolites

In preliminary experiments we used the active metabolite of vitamin D_3, $1,25(OH)_2D_3$, at different concentrations ranging from 10^{-10} M to 10^{-6} M. These preliminary experiments confirmed that other D_3 metabolites not hydroxylated in carbon 1 position (e.g. 25-OH D_3: $24,25(OH)_2D_3$) have no detectable effect on T cells at concentrations less than 10^{-6} M, and that at 10^{-6} M there was a considerable (>30%) drop in cell viability after 24 hr. The $1,25(OH)_2D_3$ was dissolved in a final concentration of 0·01% ethanol: at this concentration ethanol had no effect on either cell viability or proliferation. The concentration of active metabolite present in the serum-supplemented medium was less than 10^{-12} M measured by radioimmunoassay (Clemens *et al.*, 1978). Based on these preliminary studies, we used a concentration of 10^{-7} M throughout.

Interleukin-2 assay

Tonsil T cells (5×10^5 per ml) were incubated for different time-periods in the presence or absence of mitogens and with or without 10^{-7} M $1,25(OH)_2D_3$. IL-2 production was measured by titration of the cell-free supernatant onto an IL-2-dependent cell line, CTLL (gift of Dr D. Rayner, Middlesex Hospital, London). After 34 hr culture, the cells were pulsed with ^{125}IUdR. Cultures were harvested at 40 hr. Results are expressed as a percentage of the CTLL response to a 50% concentration of a known IL-2-containing supernatant from the EL-4 cell line.

$1,25(OH)_2D_3$ receptor

The $1,25(OH)_2D_3$ receptor was measured by a whole-cell nuclear association assay. Cell suspensions were incubated for 60 min at 37° with increasing amounts (0·125–2·5 nM) of 1,25[26,27-^{3}H]dihydroxycholecalciferol (specific radioactivity 180 Ci/mmol, Amersham) in serum-free medium. The cells were then harvested by centrifugation (500 ***g*** for 2 min) and washed three times in phosphate-buffered saline. The nuclei were isolated by incubation of the cells for 5 min in a lysis buffer containing 0·25 M sucrose and 1% Triton × 100 followed by centrifugation at 1000 ***g*** for 5 min. Nuclear pellets were resuspended in 0·2 ml of PBS and 1·0 ml of 95% ethanol, transferred to a scintillation vial, and 8 ml of solubilizing fluor (Tan 199, Packard, Lombard, IL) added. The radioactivity was measured by counting for 5 min each in a liquid scintillation spectrophotometer. The affinity (K_d) and number of the receptors were calculated by Scatchard analysis of the specific binding of the tritiated hormone.

RESULTS

T-cell mitogen responses

The results of a representative T-cell proliferative response to a standard mitogen are shown in Fig. 1. The cells were cultured for 96 hr with or without 0·001 mg/ml PHA and with or without 10^{-7} M $1,25(OH)_2D_3$. The background endogenous proliferation is higher in the low density (LD) T cells than in those from the intermediate (ID) and high density (HD) fractions, but after 4 days' culture in the presence of PHA the proliferative reponses are comparable, irrespective of the starting population used.

In these experiments there is a small inhibitory effect on the background proliferation of the cells without mitogen, but the most striking effect of $1,25(OH)_2D_3$ is seen in the presence of the mitogen. Irrespective of the density of T cells, after 4 days in culture there is less proliferation in the presence of the metabolite than with the mitogen alone.

In order to clarify this effect further, we investigated whether it was possible to generate a proliferative response in the presence or absence of the metabolite. Figure 2 illustrates that

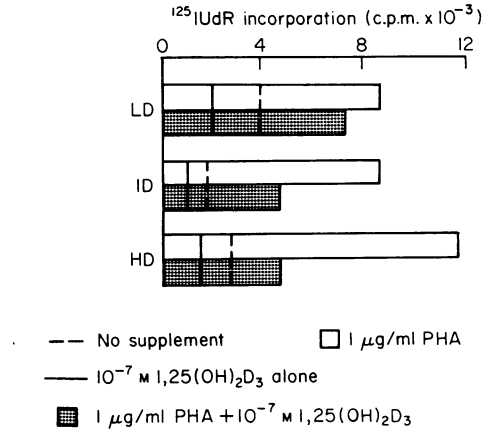

Figure 1. Effect of 1,25(OH)$_2$ vitamin D$_3$ on 96-hr PHA blast induction. Tonsillar cells were separated by density into three different subpopulations and the non-adherent cells from each subpopulation rosetted to separate off E$^+$ T cells (see Materials and Methods). Triplicate 0·1-ml aliquots of a 5 x 10^5/ml cell suspension were cultured for 96 hr with or without mitogen and with or without 10^{-7}M 1,25(OH)$_2$D$_3$. For the last 6 hr ^{125}IUdR was added to the cultures. Results of a representative experiment are shown as the mean c.p.m. radiolabel incorporation x 10^{-3}. SD for each population was less than 15%.

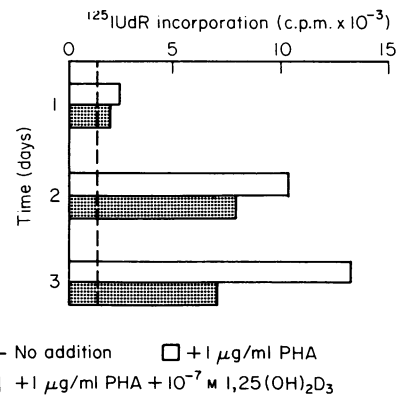

Figure 2. Effect of 1,25(OH)$_2$ vitamin D$_3$ on PHA blast induction. ID E$^+$ T cells isolated from tonsillar tissue were cultured for 24, 48 and 72 hr as outlined in Fig. 1. Six hours before harvesting the cultures ^{125}IUdR was added. Results of a representative experiment are shown as the mean c.p.m. incorporation of radiolabel x 10^{-3}. SD for each of the populations was less than 15%.

an initial activation step occurs before the 1,25(OH)$_2$D$_3$ effect is seen. Thus, blast induction is necessary first, and does occur in the presence of the metabolite. On Day 1 there is only background incorporation and the effect of the 1,25(OH)$_2$D$_3$ is negligible; on Day 2 blast induction has occurred and there is partial inhibition; and finally on Day 3 there is a 50% decrease in the response in the presence of the 1,25(OH)$_2$D$_3$.

We also examined the effect of adding 1,25(OH)$_2$D$_3$ sequentially over a 3-day period (Fig. 3). If no 1,25(OH)$_2$D$_3$ is present, there is a blast response. If the metabolite is added 24 hr after initiation of the culture, the inhibitory effect is the same as if it is present for the full time–course of the response. If the metabolite is added 48 hr after the start of the culture period, blast

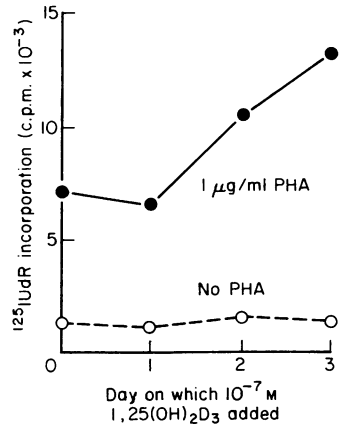

Figure 3. Addition of 1,25(OH)$_2$D$_3$ during blast induction (3-day culture period). Culture conditions were similar to those shown in Fig. 2, but the 10^{-7} M 1,25(OH)$_2$D$_3$ was added 0, 24 and 48 hr after the start of the culture period rather than at the start. Results are expressed as outlined in Fig. 2.

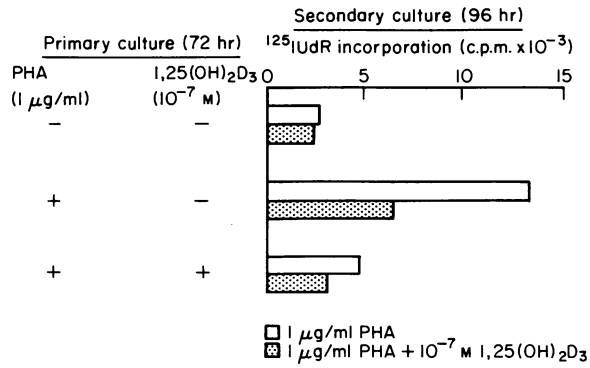

Figure 4. 5 x 10^5/ml ID E$^+$ cells were cultured for 72 hr with and without mitogen and with and without 10^{-7} M 1,25(OH)$_2$D$_3$. The cells were harvested, counted, and equal numbers of cells were recultured under the same conditions as were used in the previous experiments (Fig. 2). The radiolabel (^{125}IUdR) was added to the secondary culture at 90 hr. Results of a representative experiment are shown comparing the cells that had been precultured alone, with PHA, and with both PHA and 1,25(OH)$_2$D$_3$ added. The results are expressed as outlined previously (Fig. 2).

induction has already occurred so the metabolite will inhibit proliferation. However, this is not as marked as when the metabolite is present throughout the culture period.

Effect of 1,25(OH)$_2$D$_3$ on T-cell blasts

Since blast induction seemed to be necessary in order to demonstrate the 1,25(OH)$_2$D$_3$-mediated inhibitory pathway, we also carried out experiments on cells that had been precultured in the presence of mitogen, and in the presence or absence of the metabolite. Figure 4 shows the response of these precultured blasts taken from the ID E$^+$ layer. Cells were harvested 3 days after initiating the culture and the number of viable cells were counted. Equal numbers of viable cells were then re-incubated either with or without mitogen and with or without 1,25(OH)$_2$D$_3$. The unstimulated control shows no proliferation.

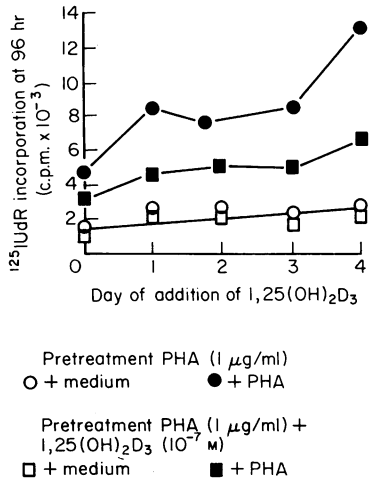

Figure 5. PHA blasts: addition of $1,25(OH)_2D_3$ during period of culture. The same culture conditions for the primary responses were used as in Fig. 4. However, instead of adding 10^{-7} M $1,25(OH)_2D_3$ at the start of the secondary culture, the metabolite was added at times 0, 24, 48 and 72 hr after the start of the secondary culture period. Radiolabel was added at 90 hr after the initiation of the secondary culture; results are expressed as outlined previously (Fig. 2).

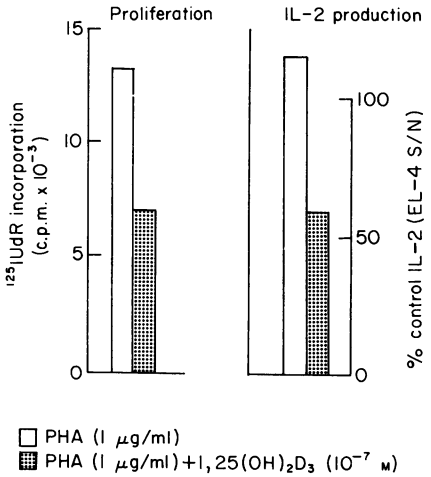

Figure 6. Comparison of T-cell proliferation with IL-2 production. Comparative results of the proliferation and IL-2 synthesis assays using the same T-cell pool. On the left-hand side (proliferation) is a standard isotope incorporation assay (for method, see legend to Fig. 2). On the right-hand side (IL-2 production) the same T cells were used with mitogen and with or without $1,25(OH)_2D_3$, but in tissue culture flasks at 5×10^5/ml rather than in microtitre wells. After 72 hr the supernatants were harvested and assayed for IL-2 synthesis. Results are expressed as the capacity to induce proliferation of an IL-2-dependent T-cell line, CTLL, compared to a standard known IL-2-containing supernatant from the EL-4 cell line which was taken as 100%.

The PHA blasts are actively proliferating after a total of 7 days in culture. Adding $1,25(OH)_2D_3$ to the blasts is inhibitory to the T-cell blast proliferation. However, the most dramatic effect is that seen with PHA blasts that were preincubated with $1,25(OH)_2D_3$. These cells are refractory to subsequent stimulation, irrespective of whether $1,25(OH)_2D_3$ is added in the second phase or not.

Figure 5 demonstrates this refractory property of the pretreated T cells in another way. In this type of experiment we added the $1,25(OH)_2D_3$ on consecutive days during the secondary culture. The cells that had been precultured in the metabolite remained relatively resistant to further stimulation. The proliferation paralleled that seen with blasts generated in the absence of the metabolite but remained consistently less than that seen when no metabolite is added. For these preinduced blasts, unlike the primary T-cell population, even adding the $1,25(OH)_2D_3$ during the final 24 hr of a 96 hr period is sufficient to cause a decrease in proliferation.

Interleukin-2 production

In parallel with the direct T-cell proliferative responses, we examined whether the effect of $1,25(OH)_2D_3$ was related directly to IL-2 production in these experiments. A representative experiment is shown in Fig. 6. E^+ cells incubated with 10^{-7} M $1,25(OH)_2D_3$ produce less IL-2 than those that are stimulated with mitogen alone.

$1,25(OH)_2D_3$ receptors

Blast induction is necessary before $1,25(OH)_2D_3$ receptors can be identified in human peripheral blood T cells, but there is no previous evidence to clarify whether all T cells that have migrated into the tissues will express this receptor, or whether activation is still required. Figure 7 shows an experiment that

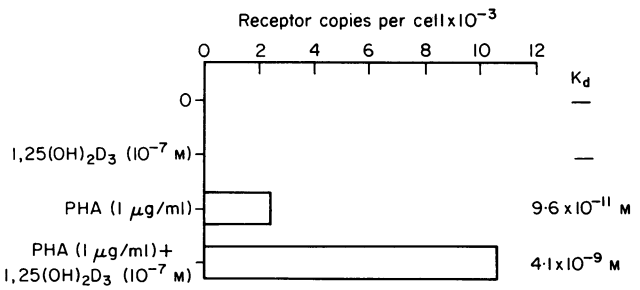

Figure 7. Induction of $1,25(OH)_2$ vitamin D_3 receptors. ID E^+ T cells (5×10^5/ml) were cultured for 72 hr with and without mitogen and with and without 10^{-7} M $1,25(OH)_2D_3$ as outlined in the text. The cells were harvested and counted. Equal numbers of viable cells were incubated for 60 min with increasing amounts of radiolabelled $1,25(OH)_2D_3$, the cells harvested and washed, and the lysed nuclear pellets resuspended in PBS/ethanol (1·0 ml). The radioactivity incorporated was measured and the affinity and number of receptors were calculated by Scatchard analysis of binding of tritiated hormone. Results are expressed as the number of receptor copies per cell $\times 10^{-3}$ and as K_d.

confirms that activation is also necessary for the T cells derived from the tissues. In addition, the experiment also indicates that the induction of the receptor is itself increased considerably in the presence of the metabolite. The control population shows that culture with the metabolite alone does not have an effect.

DISCUSSION

The role of T cells in human immune responses has generally

been investigated using peripheral blood as the source of the test population. Since many T-cell responses are mediated in local tissue sites, an important aspect of the study of immunoregulation is to examine potential similarities and differences between the peripheral (blood) and local (tissue) populations. Some of these differences may be important in the development of local (as opposed to systemic) immune responses.

In this study we describe the use of tonsillar T cells in an *in vitro* system. The results illustrate that blast induction does occur successfully in these cells. However, to devise a model of the life-cycle of a T cell within the tonsil (and probably other lymphomedullary sites), preselection is necessary to eliminate activated T cells such as the LD E^+ population.

Once the potential responder population has been characterized in the ID or HD layer, then *in vitro* methods can be used to examine their responses to signals that might occur in their own physiological environment. Recent experiments (Rook *et al.*, 1986) have confirmed that $1,25(OH)_2D_3$ is a possible endogenous product that might be available as a physiological T-cell modulator. One aspect of this is that the metabolite has a role in cell differentiation and that the cell line HL-60 could be a model precursor system to look at the formation of differentiated cells such as macrophages and other potential accessory cells (Reitsma *et al.*, 1983). Perhaps more significant for direct tissue intervention by the metabolite is that monocytes and macrophages are able to 1-hydroxylate the precursor 25-OH cholecalciferol (Cohen & Gray, 1984), and thus generate the active form of the hormone locally when required for intervention in an immune response.

This study illustrates that tonsillar T cells are not preactivated with respect to the effect of $1,25(OH)_2D_3$. Receptors for this metabolite are not present on the cells when isolated. Blast induction is a prerequisite for the metabolite to have an effect, and once blast induction has occurred the metabolite will then inhibit further expansion of the T-cell pool.

This observation is similar to the observations that have been made with peripheral blood cells (Bhalla *et al.*, 1983). Likewise, our observations with regard to IL-2 production would support the same hypothesis: namely, that the T cell does not produce its own IL-2 hormonal signal in the presence of this other hormone. However, since the metabolite is a local product from the monocytes in the tissues, and thus might be present at the site of a reaction for some considerable period, we were interested to establish whether its effect could be reversible. Thus, we used T-cell blasts induced in the presence or absence of the metabolite and investigated whether we could generate a further blast response. The results suggest that $1,25(OH)_2D_3$ has a long-lasting effect on the expansion of the T-cell pool.

One of the possible explanations for the requirement that blast induction antecedes the $1,25(OH)_2D_3$ inhibition is that receptors for the hormone are not identifiable in unstimulated T cells (Bhalla *et al.*, 1983). Thus, blast induction and receptor induction are both activation events, and the lack of receptor correlates with lack of susceptibility to the hormone. In this study we have shown that T cells in tonsil lack receptor before activation, and that the effects on IL-2 synthesis and cell proliferation are only seen when receptor expression has been induced. Put another way, it is apparent that $1,25(OH)_2D_3$ receptor induction is not necessary before the T cell migrates from the peripheral blood to the tissues.

Furthermore, in parallel experiments in which we incubated the T cells with substrate as well as mitogen, we were able to show clearly that there is hyperinduction of receptor within these cells. This implies that the active form, $1,25(OH)_2D_3$, is itself acting as a stimulus for the synthesis of its own receptor and hence acting as mediator to maintain a negative signal during lymphoid stimulation.

Together with the proliferation studies and the known ability of the monocyte to synthesize this metabolite, our results support the notion of $1,25(OH)_2D_3$ acting within the immune system as an autoregulatory hormone that has the effect of maintaining a constant T-cell population after activation. If $1,25(OH)_2D_3$ is such an effective local regulator of the immune response for T cells in human tissues, then this observation might be important in some disease states. For example, recent studies have suggested that macrophages from sarcoid patients are particularly active as synthesizers of the metabolite (Adams & Gacad, 1985), and this may lead to increased circulating levels of $1,25(OH)_2D_3$ in the peripheral blood (Fraher, unpublished observation) Thus, $1,25(OH)_2D_3$ may play a key role in the pathogenesis of the focal immune response reaction known as a granuloma. Previous theoretical approaches to the topic of granulomas have concentrated on the nature of the signals that induce the macrophage aggregation and transformation leading to the distinctive monocyte-derived aggregates (Hahn & Kaufmann, 1981), and it is these aggregates that distinguish between granulomatous and other forms of the chronic inflammatory response. Our findings emphasize an alternative: that the key different event may not be the macrophage activation but rather the inhibition of chronic polyclonal inflammatory T-cell proliferation by a macrophage product. Thus, the typical morphology of the granulomatous reaction is visualized as consisting of an epithelioid cluster of secretory macrophages because the T-cell expansion is inhibited, and this inhibition is due to products of the monocytes lineage itself. This model for the pathogenesis of a granuloma would emphasize the possible autoregulatory role that local products such as $1,25(OH)_2D_3$ may play in the evolution of human disease patterns arising in solid tissue, as seen in the mycobacterioses and sarcoidosis.

REFERENCES

ADAMS J.S. & GACAD M.A. (1985) Characterisation of 1-alpha-hydroxylation of vitamin D_3 sterols by cultured alveolar macrophages from patients with sarcoidosis. *J. exp. Med.* **161**, 755.

BHALLA A.K., AMENTO E.P., SEROG B. & GLIMCHER L.H. (1984) 1,25-Dihydroxyvitamin D_3 inhibits antigen-induced T cell activation. *J. Immunol.* **133**, 1748.

CLEMENS T.L., HENDY G.N., GRAHAM R.F., BAGGIOLINI E.G., USKOKOVIC M.R. & O'RIORDAN J.L.H. (1978) A radioimmunoassay for 1,25-dihydroxycholecalciferol. *Clin. Sci. mol. Med.* **54**, 329.

COHEN M.S. & GRAY T.K. (1984) Phagocytic cells metabolize 25 hydroxyvitamin D_3 *in vitro*. *Proc. natl. Acad. Sci. U.S.A.* **81**, 931.

GEHA R.S. & MERLER E. (1974) Response of human thymus-derived (T) and non-thymus-derived (B) lymphocytes to mitogenic stimulation *in vitro*. *Eur. J. Immunol.* **4**, 193.

HAHN H. & KAUFMAN S.H.F. (1981) The role of cell mediated immunity in bacterial infections. *Rev. inf. Dis.* **3**, 1221.

PARISH C.R. & MCKENZIE I.F.C. (1978) A sensitive rosetting method for detecting subpopulations of lymphocytes which react with alloantisera. *J. immun. Meth.* **20**, 173.

PROVVEDINI D.M., TSOUKAS C.D., DEFTOS L.J. & MANOGLAS S.C. (1983)

1,25-dihydroxyvitamin D: receptors in human leukocytes. *Science*, **221,** 1181.

REITSMA P.H., ROTHBERG P.G., ASTRIN S.M., TRIAL J., BAR-SHAVIT Z., HALL A., TEITELBAUM S.L. & KAHN A.J. (1983) Regulation of *myc* gene expression in HL-60 leukaemia cells by a vitamin D metabolite. *Nature (Lond.)*, **306,** 492.

ROOK G.A.W., STEELE J., FRAHER L., BARKER S., KARMALI R., O'RIORDAN J. & STANFORD J. (1986) Vitamin D_3 gamma interferon, and control of proliferation of *Mycobacterium tuberculosis* by human monocytes. *Immunology*, **57,** 1.

SUNSHINE G.H., KATZ D.R. & FELDMAN M. (1980) Dendritic cells induce T cell proliferation to synthetic antigens under Ir gene control. *J. exp. Med.* **152,** 1817.