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Recombinant interleukin-4 promotes expression of the CD25 (Tac) antigen at the plasma membrane of high-density human tonsillar B lymphocytes

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SUMMARY

High-density human B lymphocytes were prepared from tonsillar mononuclear cells by depletion of adherent cells and T lymphocytes, followed by discontinuous density gradient centrifugation. The B cells were analysed for the levels of expression of the CD25 (Tac) antigen marker by flow cytometry following culture with a variety of cytokines. IL-4 could induce elevated levels of CD25 on high-density, putatively resting B lymphocytes in a dose-dependent fashion. Expression of CD25 at the B-cell surface could not be promoted by interleukin-2 (IL-2), interferon-gamma (IFN-γ) or by a crude preparation of B-cell growth factor 2 (BCGF-2). Mitogenic challenge of the B cells with pokeweed mitogen (PWM) and a combination of phorbol ester and calcium ionophore were similarly ineffective, although a small increase in CD25 expression could be detected when the B cells were cultured with phytohaemagglutinin A (PHA). The ability of IL-4 to promote CD25 expression was abolished by the presence of IFN-γ in the culture. Titration experiments suggested that the amount of IL-4 required to produce a half-maximal increase in CD25 expression was approximately 40 U/ml; this is considerably greater than the 8–10 U/ml required to produce the equivalent effect on CD23 expression. The ability of IL-4 to promote CD25 expression in the high-density B-lymphocyte population was apparently independent of proliferation of the cells. IL-4 could not promote Tac expression on high-density T cells prepared from the same tissue source.

INTRODUCTION

Interleukin-4 (IL-4) is a T-cell-derived cytokine that modulates the immunological responsiveness of human B lymphocytes. Responses that are positively up-regulated by IL-4 include increased expression of the CD23 antigen (Kikutani *et al.*, 1986a; DeFrance *et al.*, 1987), a cell surface structure which, upon subsequent cleavage, may function as an autocrine growth factor for B cells (Swendeman & Thorley-Lawson, 1987; Gordon & Guy, 1987), and promotion of isotype switching to IgE in uncommited B cells (Pene *et al.*, 1988). The human IL-4 molecule is a 140 amino acid protein (inclusive of leader peptide) with three potential *N*-glycosylation sites (Noma *et al.*, 1986), and binds to a single population of high-affinity, non-cooperative receptors on IL-4-responsive cells, including B cells (Park *et al.*, 1987; Cabrillat *et al.*, 1987). Many of the IL-4-driven effects noted in B lymphocytes are antagonized by inteferon-gamma (IFN-γ) (Pene *et al.*, 1988), via a mechanism that does not involve competitive inhibition of IL-4 binding to its complementary receptor. Finally, the ability of IL-2 to support the proliferation of activated B cells is apparently inhibited by IL-4 (Jelinek & Lipsky, 1988).

The human receptor for IL-2 is known to exist as three populations, each with characteristic affinities for IL-2 (Smith, 1988). The IL-2 receptor complex comprises, minimally, two glycoprotein chains and it is the expression and interaction of these component chains which provides the structural basis for receptor affinity. The best characterized of the two component chains of the IL-2 receptor is the 55,000 MW transmembrane glycoprotein originally defined by the Tac antibody (Leonard *et al.*, 1982), and now defined by the CD25 antibody cluster. The second component of the IL-2 receptor, defined by chemical cross-linking studies using radioactive IL-2 (Tsudo *et al.*, 1986), is a 75,000 MW transmembrane glycoprotein. The low ($K_d = 10$ nM) and intermediate ($K_d = 1$ nM) affinity populations of IL-2 receptor are explained in terms of binding of IL-2 solely to the CD25 structure or solely to the 75,000 MW chain, respectively. The high-affinity ($K_d = 10$ pM) population is explained in terms of IL-2 interacting with an heterodimeric receptor complex containing one CD25 molecule and one 75,000 MW molcule (Robb *et al.*, 1987; Teshigawara *et al.*, 1987; Smith, 1988).

In this study, we sought to address the influence of IL-4 upon the level of the expression of the IL-2 receptor in high-density

Abbreviations: AET, aminoethylisothiouronium bromide; BCGF-2, B-cell growth factor 2; FITC, fluorescein isothiocyanate; IFN-γ, interferon-gamma; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; PE, phycoerythrin; PHA, phytohaemagglutinin A; PI, propidium iodide; PMA, phorbol-12-myristoyl-13-acetate; PWM, pokeweed mitogen; SRBC, sheep red blood cells.

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human tonsillar B lymphocytes. Flow cytometric analysis indicates that culture of such lymphocytes with IL-4 promotes the level of expression of the CD25 (Tac) antigen at the surface of the B cells. These data are of some interest in view of recent results which suggest that, in mouse B cells, the expression of the CD25 equivalent molecule is controlled by IL-5 rather than IL-4 (Loughnan & Nossal, 1989).

MATERIALS AND METHODS

Materials

Tonsils were obtained at surgery and were kindly provided by Mr Chandrachud, Royal Hospital for Sick Children, Yorkhill, Glasgow. Tissue culture media and fetal calf serum (FCS) were obtained from Gibco, Paisley, Renfrewshire. Percoll was purchased from Sigma (Poole, Dorset). Recombinant human IL-4 was purchased from Genzyme via A. & J. Beveridge, Edinburgh. The BCGF-2-containing supernatant was purchased from Cellular Products Inc., Buffalo, NY and the IFN-γ-containing supernatant was a generous gift from Dr D. B. Thomas, NIMR, London. The activities of IL-4 stated in this report are those reported by the supplier and are based upon the unit definition of Grabstein *et al.* (1986). All monoclonal antibody reagents were purchased from Becton-Dickinson, Abingdon, Oxon, with the exception of FITC anti-mouse IgG (Fc region specific), which was obtained from Amersham International plc, Aylesbury, Bucks from whom radiochemicals were also obtained. The MHM6 (anti-CD23) monoclonal antibody was a generous gift from Dr J. Gordon, University of Birmingham.

Lymphocyte preparation and tissue culture

B cells were prepared from a single cell suspension of freshly spilled tonsillar cells as described previously (McGarvie & Cushley, 1989). The total cell population was collected by centrifugation, then washed three times in RPMI-1640 medium lacking serum, but supplemented with 2 mM fresh glutamine, 1 mM sodium pyruvate, 10^3 U/ml penicillin and 1 mg/litre streptomycin (incomplete medium). Buffy coat cells were obtained by centrifugation on Ficoll–Hypaque (Pharmacia, Milton Keynes) and washed three times with incomplete medium. The cells were resuspended at 10^7 cells/ml of RPMI-1640 medium supplemented with 5% (v/v) heat-inactivated FCS, and cultured on plastic petri-dishes for 45 min at 37°. The non-adherent cells were harvested and subjected to two successive passages over Sephadex G10 columns. The eluted cells were washed twice in incomplete medium, resuspended at 10^7 cells/ml, and AET-treated sheep red blood cells (AET-SRBC) (Pharmacia) added to a final concentration of 1·5% (v/v). The rosettes were centrifuged over a Ficoll–Hypaque cushion, and the non-rosetted cells collected from the interface, washed three times in incomplete medium and subjected to a further cycle of AET-SRBC rosetting. The non-rosetting cells were then centrifuged over a discontinuous Percoll gradient (Radcliffe & Julius, 1982) and small resting B cells collected from the 1·08/1·09 g/ml interface. The B-cell population was washed three times prior to culture.

In experiments where T cells were enriched, a suspension of spilled tonsillar cells (10^7/ml in incomplete medium) was passed over a nylon-wool column, and the eluted cells treated with anti-HLA-DR (Sera Lab, Crawley Down, Sussex) and anti-human IgM antibodies (Sigma) in the presence of guinea-pig complement (Sera-Lab) for 45 min at 37°. The cells were washed three times with incomplete medium and subjected to Percoll density gradient centrifugation as described for B-lymphocyte preparation. Cell populations were analysed by simultaneous two-colour flow cytometry before and after AET-rosetting or antibody-mediated complement lysis to monitor enrichment for B and T cells, respectively.

Cells were cultured in RPMI-1640 medium containing 10% (v/v) FCS, 2 mM fresh glutamine and 1 mM sodium pyruvate (complete medium) supplemented with the concentrations of IL-4 and other ligands noted in the figure legends for the time periods indicated. Antibiotics were omitted from these routine cultures. After the appropriate culture interval, the cells were harvested and analysed by flow cytometry.

Flow cytometry

Simultaneous two-colour immunofluorescence analysis was performed using FITC- and PE-labelled monoclonal antibodies. All analyses of CD25 expression were performed using a FITC-labelled anti-CD25 reagent, while monoclonal antibodies to B- and T- lymphocyte-specific markers, either CD3 or CD19, were PE-labelled. Aliquots of 10^6 cells were used in each analysis, and were washed three times in ice-cold PBS containing 0·5% (w/v) BSA and 10 mM sodium azide (PBS-azide). Staining was performed on ice for 45 min, with the FITC anti-CD25 and PE anti-CD19 (or CD3) antibodies being present simultaneously, followed by further washing prior to analysis.

For staining of the CD23 marker, cells were incubated with a small aliquot of MHM6 ascites fluid for 30 min on ice, washed, and 50 μl of a 1:10 dilution of FITC anti-mouse IgG added and the mixture incubated on ice for a further 30 min. The cells were then washed three times with PBS-azide before addition of the PE-labelled anti-CD19 reagent.

Propidium iodide was added to all samples to a final concentration of 2 μg/ml immediately prior to analysis to permit exclusion of dead cells. Samples were analysed on a Becton-Dickinson FACScan flow cytometer and data were handled using the FACScan Research software program. The gating out of non-viable cells was performed by identifying those cells which showed a bright ($>10^2$ arbitrary fluorescence units) staining in the FL3 (>650 nm) detector, and preventing the acquisition of such cells in the data collection. 10^4 gated events were collected for each sample. Scoring of the percentage of B cells positive for a given marker (e.g. CD25) were calculated from FL1/FL2 two-colour dot plots. The percentage of CD25 positive B cells was calculated according to the following equation:

$$\%\ CD25^+\ B\ cells = \frac{\text{no. of } CD19^+/CD25^+ \text{ cells}}{\text{no. of } CD19^+/CD25^+ \text{ cells} + \text{no. of } CD19^+/CD25^- \text{ cells}} \times 100.$$

Proliferation assays

Cells were harvested from the bulk cultures, washed with incomplete medium and suspended at 10^7 cells/ml in medium supplemented with 20% (v/v) FCS. Aliquots of 50 μl of this suspension were placed in individual wells of 96-well microtitre trays containing ligands and/or mitogen: the final culture volume was 100 μl. Cells were pulsed for 6 hr prior to harvest with 1 μCi/

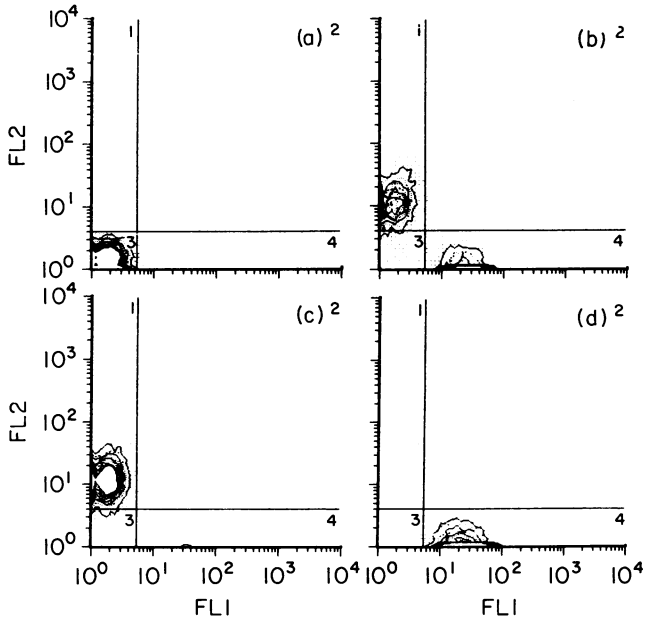

Figure 1. Characterization of lymphocyte preparations. Cells were spilled from tonsillar explants, washed and centrifuged over Ficoll-Hypaque as described in the Materials and Methods. The non-specific staining of this mononuclear cell population was assessed by addition of FITC-IgG1 and PE-IgG2 immunoglobulins to the cells and analysing the staining profile on two-colour contour graphs (a); cells staining with this reagent combination were used to define non-specific staining (quadrant 3). This cell population was also stained with FITC anti-CD3 and PE anti-CD19 reagents to determine the proportion of B and T cells in the mononuclear cell population (b). B cells were isolated by two rounds of AET-SRBC rosetting, followed by selection of high-density cells on Percoll (c), and T lymphocytes were obtained by depletion of nylon-wool adherent cells, complement-mediated elimination of B cells followed by Percoll density gradient centrifugation (d). All samples were counter-stained with propidium iodide to permit gating-out of dead cells. 10,000 gated events were collected and analysed by the Consort 30 program. The minimum level for dots was 1 event, and the contours were spaced at intervals of 10 events with maximum of 90. On each contour plot, the labelling of the axes is reported as FL1 (x-axis) and FL2 (y-axis), which refer to fluorescence from FITC and PE, respectively.

well [^{3}H]thymidine or [^{3}H]uridine. Incorporation of radioactivity was determined by liquid scintillation spectrometry.

RESULTS

Characterization of B-cell populations

The B lymphocytes used in this study were prepared from single cell suspensions of adherent cell-depleted tonsillar cells by removal of T lymphocytes by two cycles of rosetting with AET-SRBC. In the non-adherent cell-enriched tonsillar mononuclear cell population, CD3$^+$ staining cells routinely accounted for some 35–40% of the cells, with 50–60% of cells having a CD19$^+$ phenotype. In the example illustrated, the CD3$^+$ cells accounted for 39% of the total mononuclear cell population, with CD19$^+$ cells comprising 57·3% of the population, with 1·3% null staining cells (Fig. 1b). Following AET-SRBC rosetting and density gradient centrifugation, the proportion of CD3$^+$ cells (quadrant 4) was reduced to $< 5\%$ of the total population, and the CD19$^+$-staining cells accounted for approximately 95% of the non-rosetting cell population (data not shown). The inclusion of a second cycle of AET-rosetting reduced the CD3$^+$ population to less than 1% (0·6% in the example shown), with B cells (quadrant 1) accounting for $> 97\%$ of the cells (Fig. 1c). When T cells were enriched from the mononuclear cell suspension, the purified population contained approximately 2% CD19$^+$ B cells and 96·3% CD3$^+$ cells (Fig. 1d). In both purified populations non-specifically staining cells (quadrant 3) accounted for $< 1{\cdot}5\%$ of the total cells. The quadrant markers were set using FITC-IgG1 and PE-IgG2 control immunoglobulins to define null or non-specific staining (Fig. 1a).

Two-colour analysis of CD25 expression on B lymphocytes

Culture of high density tonsillar B lymphocytes, prepared as described above, with IL-4 resulted in a small but consistent increase in CD25 expression in one-colour analyses (data not illustrated). B lymphocytes were cultured with 400 U/ml IL-4 and harvested at the time intervals indicated in the figure. The cells were then treated simultaneously with FITC anti-CD25 and PE anti-CD19 reagents, and the staining patterns visualized on two-colour plots (Fig. 2). The data illustrate that the number of CD19$^+$ cells becoming positive with respect to CD25 (i.e. cells in quadrant 2 of the plots) increases with time, rising from approximately 2% in the freshly prepared resting population (Fig. 2a) to some 16·5% after 24 hr (Fig. 2e) and peaking at approximately 27–29% after 60–72 hr in culture with the cytokine (Fig. 2h, i). These data are consistent with the interpretation that the increase in CD25 expression is localized to the B-cell population and cannot be accounted for in terms of an effect of IL-4 upon the residual T lymphocytes in the cell population exposed to the cytokine.

Cytokine specificity of CD25 induction

The data of Fig. 2 indicate that IL-4 can promote expression of CD25 upon high-density tonsillar B lymphocytes. The ability of other cytokines and mitogens to promote this effect was studied. Figure 3 illustrates the increase in CD25-positive B cells as a function of time in culture with defined stimuli; Fig. 3c,d show the equivalent data for CD23 expression in the same experiment. The data illustrate that only IL-4 could promote high levels of CD23 expression in the high-density B-cell population; the use of IFN-γ, crude BCGF-2, IL-2, mitogens such as PHA or PWM or the combination of calcium ionophore (A23187) and phorbol ester (PMA), as stimuli all failed to increase CD23 expression (Fig. 3c,d). A similar trend of data is evident for the effects of the same stimuli upon expression of CD25 in the B-cell population. Thus, only IL-4 and, to a lesser extent, PHA were capable of promoting CD25 expression in the B cells (Fig. 3a,b).

Perhaps the most noteworthy feature of the data of Fig. 3 is the ability of IFN-γ to inhibit the IL-4-driven increase in CD25 expression in the B cells (Fig. 3a). This particular item of data suggests that the up-regulation of CD25 by IL-4 is specific to that lymphokine, since IFN-γ is known to block many of the effects of IL-4 including, for example, CD23 expression, a phenomenon also noted in these experiments (Fig. 3c).

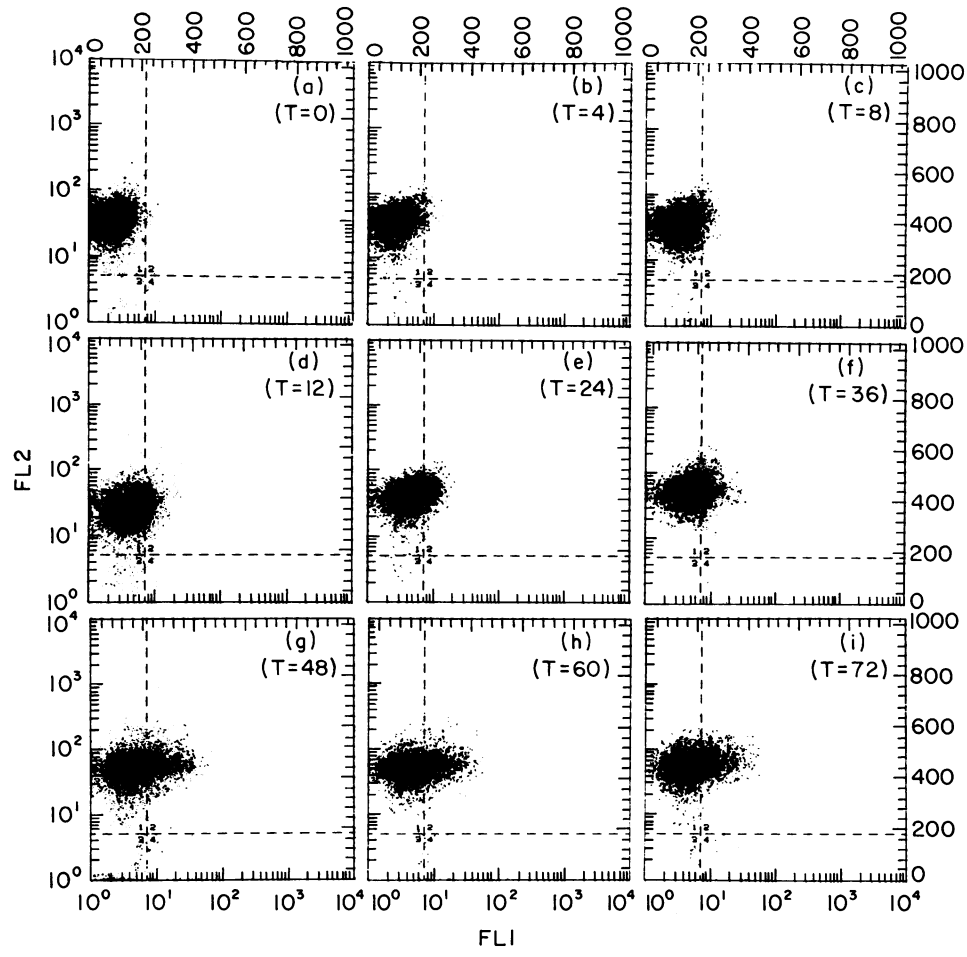

Figure 2. IL-4 promotes CD25 expression on tonsillar B cells. High-density B cells were prepared as described in the legend to Fig. 1, and cultured for the indicated intervals (T) with 400 U/ml IL-4 in complete medium. Cells were harvested at the appropriate time, washed and stained simultaneously with FITC anti-CD25 and PE anti-CD19 reagents. Dead cells were excluded from the data collection by propidium iodide counter-staining. 10,000 gated events were collected for each sample, and analysed using the FACScan Research Software program. Quadrant markers were set using FITC- and PE-labelled IgG1 and IgG2 immunoglobulins. As in Fig. 1, the *x*-axis represents green fluorescence (FITC) and the *y*-axis depicts red fluorescence (PE).

Dose-dependence of CD25 induction in tonsillar B cells

The data of Figs 2 and 3 suggest that IL-4 can promote an increase in the level of CD25 present at the membranes of high-density human tonsillar B cells. Figure 4 illustrates the effect of increasing IL-4 concentration upon the number of $CD19^+$ B cells which become $CD23^+$ or $CD25^+$ as a function of time. The increase in expression of both markers exhibited clear dose-dependence; thus, the number of B cells becoming positive for the marker of interest increased in proportion to the concentration of IL-4. The magnitude of the CD23 response, in terms of the percentage of B cells which become positive for the particular marker under analysis, was consistently considerably greater than that observed for CD25. Thus, only 5 units/ml of IL-4 were required to render 25% of the B cells positive for CD23 (Fig. 4b), whereas some 400 U/ml of cytokine were required to generate a similar number of $CD25^+$ B cells (Fig. 4a). Indeed, the data indicate that $<30\%$ of the B cells were capable of displaying an increase in CD25 expression, as suggested by the fact that no further substantial increase in $CD25^+$ B cells was noted with IL-4 concentrations up to and including 1000 U/ml (Fig. 4a).

In the context of the experiments presented in Fig. 4, the maximum number of cells which showed positive response to IL-4 was 63% for CD23 and 28% for CD25. The amount of IL-4 necessary to effect half-maximal increases in the expression of CD23 and CD25 in the B-cell population was estimated from semi-log plots of IL-4 concentration versus the percentage of cells positive for the desired marker (not illustrated). Taking the end-point of the culture (i.e. 72 hr) the values for CD23 expression and CD25 expression were estimated as 8 U/ml and 40 U/ml, respectively. The data suggest that a higher concentration of IL-4 was required to elicit a response at the level of increased CD25 expression than for enhanced CD23 expression.

Effect of cytokines upon nucleic acid biosynthesis in B lymphocytes

The capacity of the B cells to synthesize DNA and RNA in

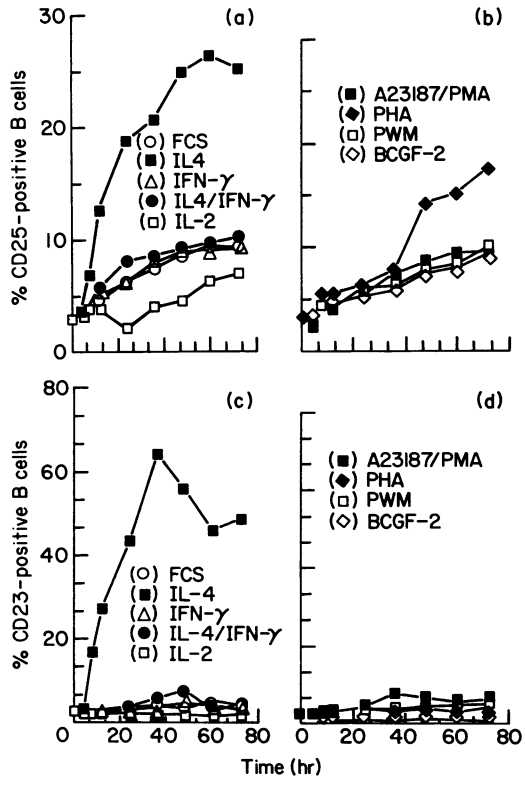

Figure 3. CD23 and CD25 expression on stimulated tonsillar B lymphocytes. B cells were cultured with stimulatory ligands for the indicated time periods as follows. For cytokines (a and c): 400 U/ml IL-4 (■), 10^3 U/ml IFN-γ (△), IL-4 plus IFN-γ simultaneously (●), 10 U/ml IL-2 (□) and 10% (v/v) BCGF-2 supernatant (b and d), harvested at the time-points indicated and stained for CD23 or CD25 positivity. For mitogens (b and d): 1 μg/ml PHA, 2 μg/ml PWM and 10 ng/ml PMA plus 1 μM A23187. The symbols are as depicted on the figure itself. All cells were also stained with PE-anti-CD19 reagents. The data were based on collection of 10,000 gated events, and analysis was performed using FACScan Research software. The data for CD25 expression are presented in (a) and (b), and the equivalent analysis of CD23 levels is shown in panels (c) and (d).

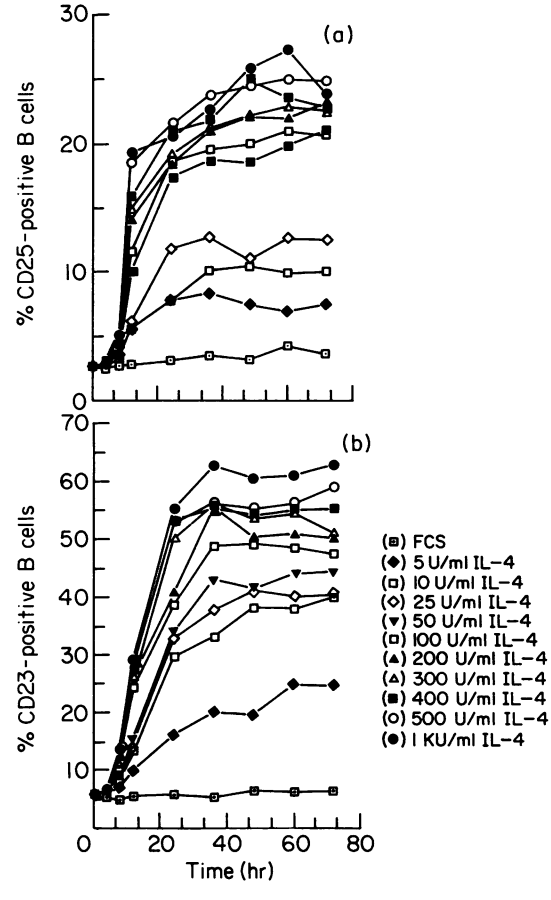

Figure 4. Effect of IL-4 concentration upon expression of CD23 and CD25 by tonsillar B lymphocytes. High-density tonsillar B lymphocytes were cultured with a three-log range of IL-4 concentrations, and samples withdrawn at the intervals indicated on the figure. The harvested cells were washed, then stained for CD19 positivity together with either CD23 or CD25 antigens. Data are based on 10,000 gated events. (a) Shows the data for CD25-positive cells, and (b) illustrates the equivalent data for CD23 expression. The symbols for each concentration employed are illustrated as the right hand edge of the figure.

response to the ligands added as stimulants was investigated (Fig. 5). DNA synthesis was estimated in a thymidine incorporation assay, and the data suggested that only the mitogen PHA and the mixture of PMA and A23187 could induce proliferation in the B-cell population, with a plateau of thymidine uptake being reached after 48 hr (Fig. 5b). None of IL-4, IL-2, or IFN-γ could promote this effect (Fig. 5a). PWM was similarly ineffective. A similar analysis of RNA synthesis again demonstrated that PHA and the PMA/A23187 combination was effective in promoting elevated uridine uptake by the B cells. In these analyses, however, IL-4 was also capable of promoting a modest elevation in RNA synthesis; this was approximately one third of the level promoted by PHA and PMA/A23187 (Fig. 5c and d). These data, taken together with those of Figs 2 and 3, suggest that the IL-4-driven increase in expression of CD25 may be independent of B-lymphocyte proliferation.

Two-colour analysis of residual T lymphocytes

The two-colour immunofluorescence study was extended to assess the effect of IL-4 upon CD25 expression in the $CD3^+$ population (Fig. 6). High-density T cells were enriched from crude tonsillar mononuclear cells, as described earlier (Fig. 1d), and cultured with the indicated cytokines at concentrations identical to those employed for B lymphocytes. Cells were harvested and subjected to simultaneous two-colour analysis using FITC anti-CD25 and PE anti-CD3 reagents. Quantification of CD25-positive cells was performed as detailed above. IL-4 was not capable of inducing greatly increased levels of CD25 at the surface of the high-density T-cell population (Fig. 6a). While a rise in $CD25^+$ cells was noted in the population exposed to the cytokine, the magnitude of this response was very similar to that induced by simple culture in serum-containing medium. Excellent promotion of CD25 was obtained when the T cells were polyclonally stimulated with mitogenic agents (Fig. 6b).

DISCUSSION

The principal finding of this report is that recombinant IL-4 can promote an increase in the level of the CD25 (Tac) antigen

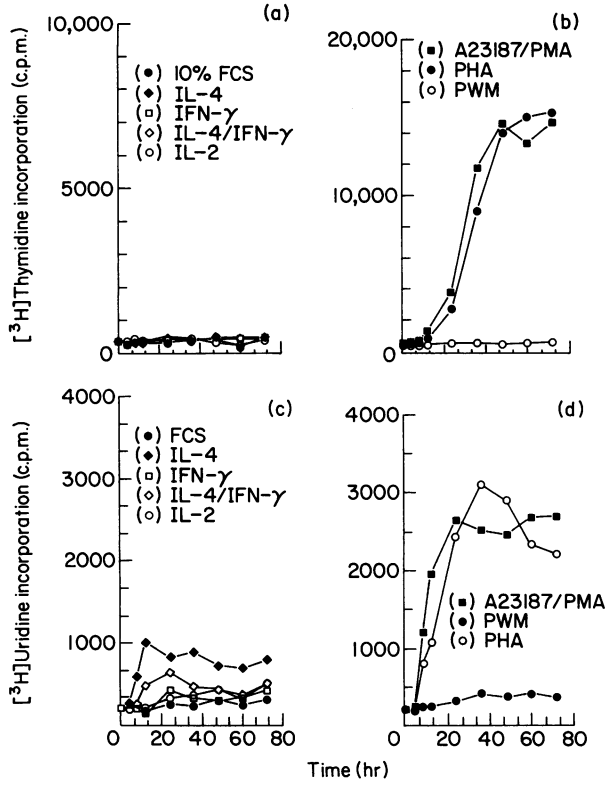

Figure 5. DNA and RNA synthesis by stimulated tonsillar B lymphocytes. Aliquots of lymphocytes were harvested from the bulk cultures stimulated with cytokines or mitogens at 6 hr prior to the time-points indicated on the *x*-axis. The concentration of stimulatory agents used was identical to that described in the legend to Fig. 3. The cells (10^6 per well) were washed then pulsed with 1 μCi of either [^{3}H]methyl thymidine (a and b) or [^{3}H]uridine (c and d) for 6 hr at 37°. The cells were harvested and incorporation of radioactivity measured by liquid scintillation spectrometry. All values reported are the means of three replicates. The symbols used for each treatment are shown on the figure.

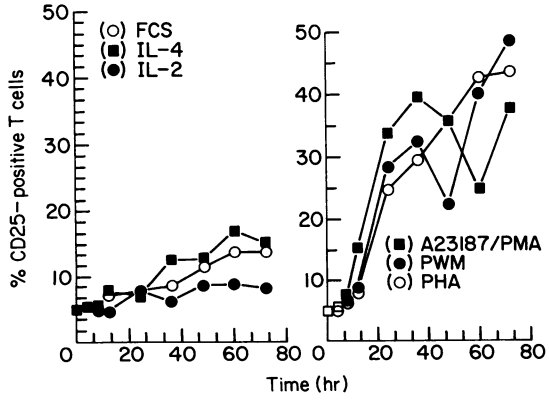

Figure 6. IL-4 fails to induce CD25 expression in CD3-positive cells. High-density tonsillar T lymphocytes were prepared as described in the Materials and Methods cultured with either IL-4, PMA plus A23187, PWM, PHA or in serum-containing medium only, and then simultaneously stained with FITC anti-CD25 and PE anti-CD3 at the time-points indicated. The data are based on collection of 10,000 gated events. (a) Illustrates the data obtained using cytokines as stimulatory agents, while (b) shows the equivalent data when mitogens were employed.

expressed at the plasma membranes of high-density human tonsillar B lymphocytes in a dose- and time-dependent manner. This effect is apparently specific to IL-4 since none of IL-2, IFN-γ or BCGF-2 could elicit a similar response in the B-cell population. Further support for the hypothesis that CD25 induction on tonsillar B cells is specific to IL-4 is provided by the observation that IFN-γ could prevent the IL-4-driven up-regulation of CD25 expression. IL-4 could not induce elevated CD25 expression upon high-density T lymphocytes.

The data of this report suggest that the control of CD25 expression in human B lymphocytes is regulated by the interaction of IL-4 with its complementary receptor. This is sharp contrast to the recent data addressing a similar question in murine B lymphocytes (Loughnan & Nossal, 1989). In the murine system, expression of the CD25-like structure appears to be regulated by IL-5, while IL-4 controls expression of the 75,000 MW component of the IL-2 receptor. How might this apparent discrepancy be explained? Firstly, the properties of the cell populations under evaluation must be considered. While every effort has been made to purify resting B cells, the possibility cannot be unequivocally ruled out that the IL-4-driven up-regulation of CD25 expression that is observed is a response unique to a subset of B cells that has already received an activatory signal. Similarly, in the murine B-cell model (Loughnan & Nossal, 1989), pre-stimulation of the B cells with an EL-4 thymoma-conditioned medium could have delivered a partial activatory signal that was subsequently magnified by exposure to IL-4. It is possible that genuinely quiescent B lymphocytes may not be capable of responding to IL-4 by promoting cell surface levels of CD25.

Secondly, murine and human B cells may respond in different ways to IL-4. For example, IL-4 can inhibit IL-2-dependent proliferative response in human B cells (Jelinek & Lipsky, 1988), while no similar reports of such a phenomenon are available in the murine B-cell models. The contribution of other cytokines to up-regulation of CD25 appear to be negligible; none of IL-2, IFN-γ or BCGF-2 was capable of generating an increase in CD25 expression. It should be stressed, however, that the cytokine activity present in the commercial BCGF-2 used in the experiments documented here would be more consistent with low molecular weight BCGF activity (Sharma *et al*, 1986) rather than with IL-5 activity. A consideration of the role of IL-5 in human and murine B-cell responses leads to the final point which may explain the difference between the data presented here and the results in the murine splenic B-cell model. That is, the ability of human IL-5 to function as a cytokine with growth promoting effects on B cells is controversial. Unlike the murine IL-5, which has excellent progression factor activity for B cells (O'Garra *et al.*, 1986), the human equivalent shows only eosinophil differentiation factor activity and has, so far, failed to display convincing effects on human B cells (Clutterbuck *et al.*, 1987; Sanderson, Campbell & Young, 1988). If human IL-5 proves to have no effect upon B lymphocytes, then other candidate cytokines must be identified which might regulate the expression of IL-2 receptor components in the human B-cell compartment. Our results suggest that a thorough investigation of the properties of IL-4 in this regard might prove valuable. It will, in any case, be interesting to elucidate the effect of recombinant human IL-5 upon the expression of IL-2 receptor components in human B lymphocytes.

The question of responses of small populations of B cells

within the cells regarded as 'resting' may provide a further important clue to the paradox. It has been suggested that the resting or G0 compartment of B lymphocytes may comprise a minimum of three distinct stages (Walker *et al.*, 1986; Gordon & Guy, 1987). It is possible that B cells residing in one of these three functional compartments responds to IL-4 by up-regulation of CD25 expression. Thus, even at very high IL-4 levels (1000 U/ml), only a quarter of the resting B cells responded by up-regulating CD25. In the case of CD23, however, some 70% of the cells were responding at this level. This observation raises the possibility that the response pattern observed may be a reflection of two mutually exclusive subsets of B cells which are responding to IL-4 in unique ways. If this proves to be correct, then the intriguing question of the nature of the B cells in the two responding populations must be addressed. Given that the only B cells which can respond to IL-4 synthesis of CD23 are mIgM$^+$ B cells (Kikutani *et al.*, 1986b), it is tempting to speculate that the B-cell subset which up-regulates CD25 in response to IL-4 could represent resting B lymphocytes committed to the biosynthesis of other immunoglobulin isotypes, i.e. resting memory B cells. The ability of human B lymphocytes in distinct phases of the G0 phase of the cell cycle and of B cells committed to synthesis of Ig isotypes other than IgM to respond to IL-4 by up-regulation of CD25 expression is currently under investigation.

The mechanism by which increased levels of CD25 on the cell surface are achieved must be addressed. Two simple explanations can be advanced. Firstly, the interaction of IL-4 with its receptor may cause *de novo* synthesis of the CD25 antigen polypeptide and its subsequent transport to the plasma membrane, or secondly, IL-4 may mobilize a pre-existing intracellular pool of CD25 molecules onto the B-cell surface. The data of this report would be consistent with an effect on gene expression rather than on mobilization of a cytokine-sensitive pool of pre-synthesized CD25. Thus, analysis of the time–course of expression of CD23 and CD25 antigens in response to IL-4 treatment are broadly similar, with no substantial increase in the level of either antigen until > 12hr of culture. For the hypothesis that IL-4 causes translocation of pre-formed CD25 to the membrane to be tenable, a rapid increase in CD25 expression, measurable at 2–4 hr after the initiation of the cytokine-stimulated cultures, should have been observed. This was not the case (Figs 2 and 4).

Finally, a monoclonal antibody with specificity for the 75,000 MW component of the human IL-2 receptor has recently been reported (Takeshita *et al.*, 1989), and it will be of great interest to discern the effect of IL-4 (and IL-5) upon the ability of human B cells to modulate the expression of the determinant recognized by this reagent. This report has concentrated upon a flow cytometric evaluation of CD25 expression in IL-4-treated B lymphocytes and, therefore, the data relate only to the expression of the 55,000 MW subunit of the IL-2 receptor family. Clearly, the effect of IL-4 upon the spectrum of IL-2 receptor affinities displayed upon the human tonsillar B lymphocyte remains to be evaluated.

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