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Functional and phenotypic analysis of human T-cell clones which stimulate IgE production *in vitro*

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SUMMARY

Peripheral blood mononuclear cells (PBMC) from a patient suffering from the hyper IgE syndrome were used to generate phytohaemagglutinin (PHA)-expanded T-cell clones (all $CD4^+$, $CD8^-$, $CD23^-$). A selection of the clones was tested for their ability to help IgE secretion by culturing with normal B cells in the presence of solid-phase antibody to CD3. Supernatants were harvested on Day 7 and assayed by ELISA for IgE, IgG and IgM. Lymphokine secretion by the clones was assessed by culturing clones for 24 hr with solid-phase antibody to CD3 followed by assay of the supernatants for IL-2, IL-4 and interferon-gamma (IFN-γ) production. In addition, clones were analysed by flow cytometry for CDw29 and CD45R expression. Initial experiments with seven clones indicated that those clones that could help IgE secretion also stimulated optimal IgG and IgM responses. All clones appeared to secrete IL-2, IL-4 and IFN-γ, although the amounts of each varied. These results confirm recent findings that human T-cell clones do not fall into Tinf (Th1) and Th (Th2) type subsets as described in the mouse. There was no clear correlation between the lymphokines secreted by the clones and their capacity to help IgE production. However, the helper function of the clones for all isotypes, including IgE, appeared to be related to the level of expression of the surface antigen CDw29.

INTRODUCTION

Immediate hypersensitivity reactions in allergic patients result from the elevation of allergen-specific IgE antibody (Ishizaka & Ishizaka, 1975). In non-allergic individuals, IgE levels are tightly controlled by suppressive mechanisms involving T lymphocytes (Ishizaka, 1984; Geha, 1984). In the past it has proved difficult to establish human IgE responses *in vitro* due to a number of factors, including this inherent suppression. Recent advances in human lymphocyte culture have led to the use of cloned T cells in helper assays with B cells from normal or atopic donors. Since the clones are of the helper-inducer phenotype, suppressive T cells are no longer present in the culture system. Different T-cell activating stimuli have been used, namely alloantigens on the responding B cells (Lanzavecchia & Parodi, 1984; Leung *et al.*, 1986), mitogens, soluble antigens or antibodies to CD3 (Nutman *et al.*, 1985; Zimmerman *et al.*, 1986; Del Prete *et al.*, 1986; Romagnani *et al.*, 1987). As an extension of these approaches, we have used T-cell clones from a patient suffering from the hyper IgE syndrome, a condition in which the disregulation of this isotype leads to very high serum IgE levels. We wanted to establish whether these clones could help B cells from normal donors to produce IgE, and whether or not this correlated with the lymphokines secreted by each clone. Both IL-4 and IFN-γ can regulate IgE responses, with IL-4 promoting and IFN-γ inhibiting production of this isotype (Coffman & Carty, 1986; Snapper & Paul, 1987; Yokota *et al.*, 1988). In the mouse, these two lymphokines are produced by different $CD4^+$ T-cell subsets; IL-4 by T helper (Th or Th2) and IFN-γ by T inflammatory (Tinf or Th1) cells (Mosmann *et al.*, 1987; Janeway *et al.*, 1988). This leads to the conclusion that, in the mouse, IgE responses are mediated by Th (Th2) cells. Recent findings suggest that while some human $CD4^+$ T-cell clones may fit into such subsets, other clones can secrete all three lymphokines (Umetsu *et al.*, 1988; Paliard *et al.*, 1988; Maggi *et al.*, 1988).

In addition to investigating the lymphokine secretion of T-cell clones, we examined the expression of two surface antigens, CDw29 and CD45R, on these cells to determine whether these markers could subdivide IgE helper and non-helper populations of T cells. These antigens were originally shown to divide $CD4^+$ T cells into two functional subsets: helper-inducers and suppressor-inducers, respectively (Morimoto *et al.*, 1985a, b). More recently, it has been suggested that the antigens define memory and naive T-cell subsets (Sanders, Makgoba & Shaw, 1988). We have found a significant correlation between the level of

Abbreviations: Ig, immunoglobulin; PHA, phytohaemagglutinin; ELISA, enzyme-linked immunoadsorbent assay; IFN, interferon; IL, interleukin; E, erythrocyte; AET, 2-aminoethylisothiouronium bromide hydrobromide; PBMC, peripheral blood mononuclear cells.

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expression of CDw29 and the ability of T-cell clones to provide help for immunoglobulin secretion of all isotypes, including IgE.

MATERIALS AND METHODS

T-cell clones

Peripheral blood mononuclear cells (PBMC) were separated from 20 ml blood, obtained from a 19-year-old woman suffering from the hyper IgE syndrome, by centrifugation over Ficoll–Hypaque (Pharmacia, Uppsala, Sweden). 5×10^7 PBMC were cultured for 3 days in RPMI-1640 supplemented with 10% human AB^+ serum (both from Flow Laboratories, Irvine, Ayrshire and 2 mM glutamine (Gibco, Paisley, Renfrewshire), with phytohaemagglutinin (PHA) (Wellcome, Beckenham, Kent) at 1 μg/ml. The T-cell blasts were then expanded for 10 days in medium as above but supplemented with recombinant human IL-2 (50 U/ml; Glaxo Group Research Ltd, Greenford) and lectin-free Lymphocult T (5%; Biotest Diagnostics, Frankfurt, FRG). Clones were isolated by seeding T-cell blasts at 0·3 cells/well in Terasaki plates (NUNC, Copenhagen, Denmark) in the presence of PHA and IL-2 as above, and with 10^4 autologous irradiated (6000 rads) lymphoblastoid cells. These were obtained from the patient's PBMC by culture with the Epstein–Barr virus (EBV) containing B95-8 supernatant. Cultures were examined for clonal growth after 1 week and the positives reseeded in 96- then 24-well plates (Costar, Northumbria Biologicals, Cramlington, Northumberland). Clones were maintained by weekly restimulation with PHA and autologous feeders and twice weekly feeds with IL-2.

Preparation of B cells

PBMC from normal volunteers were obtained by Ficoll–Hypaque centrifugation and incubated at 10^7/ml on ice with an equal volume of AET-treated sheep erythrocytes. Non-rosetting cell fractions (E^-) containing B cells and monocytes were obtained at the interface after removal of the sheep cells by centrifugation over Ficoll–Hypaque. E^- cells were partially depleted of monocytes by adherence in serum-free medium in plastic petri-dishes for 1 hr at 37°. Non-adherent cells were carefully collected, and are referred to here as B cells.

Culture conditions for immunoglobulin production in vitro

48-well tissue culture plates (Costar) were coated overnight at 37° with 500 ng/ml OKT3 antibody (Ortho Diagnostics, Raritan, NJ) in 200 μl carbonate buffer, pH 9·6. The following day, the antibody was removed and the wells washed twice with medium. Cultures were established with 5×10^5 B cells and 5×10^5 clonal T cells/well in a final volume of 1 ml RPMI-1640 with 10% fetal calf serum (FCS; Flow). Triplicate cultures were incubated at 37° in 5% CO_2 in air for 7 days. Supernatants were then removed and assayed for immunoglobulins as described below.

ELISA assays for IgG, IgM and IgE

IgG and IgM were assayed by a solid-phase enzyme immunoassay as follows. 96-well flat-bottomed plates (Nunc Immunoplates Type II) were incubated overnight at room temperature either with goat antibody to human IgG (Miles Scientific, Slough, Berks) at 1:500 in carbonate buffer pH 9·6, or goat antibody to human IgM (Miles) at 1:250. After three washes in phosphate-buffered saline (PBS-0·05% Tween 20 (BDH, Poole, Dorset), the plates were blocked by incubation with 1% BSA (Sigma, Poole, Dorset) in PBS for 30 min at 37°. Diluted samples from tissue culture, and dilutions of standard preparations of IgG (Sigma) or IgM (Miles) were incubated in the plates for a further 2 h at 37°. After three washed in PBS-Tween, plates were incubated with 1:500 dilutions (in PBS- 1% BSA) of alkaline phosphatase-labelled goat antibody to human IgG or IgM (Sigma) for 1 hr at 37°. To detect binding of the conjugated antibody, the wells were washed three times in PBS-Tween, then incubated with 1 mg/ml *p*-nitrophenyl phosphate (Sigma) in carbonate buffer, pH 9·8. After 20 min the colour developed was read at 405 nm on a Titertek Multiscan (Flow). The lower and upper limits of the assays were 7·8 and 1000 ng/ml, respectively, for both IgG and IgM.

IgG subclass ELISA

In some experiments, the levels of individual IgG subclasses were measured using kits for IgG1, IgG2, IgG3 and IgG4 as per manufacturers' instructions (The Binding Site, Birmingham). These results are expressed as IgG concentrations in ng/ml.

IgE ELISA

Flat-bottomed 96-well plates were coated with monoclonal antibody to human IgE (Serotec, Oxford, Oxon) diluted 1:500 in carbonate buffer and blocked with bovine serum albumin (BSA) as described above. Samples and standard (a polyclonal IgE preparation from the National Institute of Biological Standards, South Mimms, Herts were added, and incubated overnight at room temperature. A second blocking stage with BSA was used at this point to lower the background further. After three washes in PBS-Tween, a 1:250 dilution of biotinylated goat antibody to human IgE (Vector, Peterborough, Northants) was added and after 1 hr at 37°, and a further three washes, streptavidin-biotinylated horseradish peroxidase complex (Amersham International, Amersham, Bucks) was added at 1:500 dilution. After 1 hr at 37°, the plates were washed three times as before and incubated with 0·6 mg/ml *o*-phenylene diamine (Dakopatts, Glostrup, Denmark) in citrate-phosphate buffer, pH 5·0. After incubation at 37° for 20 min, the plates were read on a Titertek Multiscan at 450 nm. This assay was routinely sensitive to 75 pg/ml of IgE, and no cross-reactivity was seen when IgM, IgG or IgA was added at 50 μg/ml.

Production of supernatants from activated T-cell clones

T-cell clones, taken 7 days after previous stimulation, were washed twice and recultured at 10^6/ml in complete medium in untreated 48-well plates or in wells coated with antibody to CD3 as described above. After 24 h culture, supernatants were harvested, filtered through 0·22 μm filters (Gelman Sciences Ltd, Northampton, Northants) and either assayed immediately or stored in aliquots at −20°. Repeated freezing and thawing of the supernatants was avoided.

Assays for IL-2, IL-4 and IFN-γ

IL-2. IL-2 was detected in T-cell clone supernatants using the murine T-cell line CTLL. This cell line responds to human IL-2 but not human IL-4. CTLL cells were cultured at 10^4/well in 96-well U-bottomed plates, in a final volume of 100 μl complete medium containing 5×10^{-5} M 2-mercaptoethanol (sigma). T-cell clone supernatants were assayed in triplicate at a final concentration of 50% and the cells cultured for 48 hr.

Proliferation was assessed by adding 1 μCi [^{3}H]thymidine (Amersham) per well for the last 16 hr of culture, then harvesting the cells onto glass fibre mats and counting by liquid scintillation spectrometry.

IL-4. Human PHA blasts were obtained by culturing PBMC with 1 μg/ml PHA for 72 hr. These were washed three times and recultured in triplicate at 10^4/well as for the IL-2 assay, with test supernatants at 50%. However, IL-2 activity was blocked in these cultures by using rabbit antibody to IL-2 (Universal Biologicals Ltd, London) at 20 μg/ml, a concentration which could inhibit an excess of recombinant IL-2. In some assays, culture supernatant from a murine B-cell hybridoma, secreting neutralizing antibody to IL-4, was added to confirm the presence of IL-4 in the supernatants. The characterization of this monoclonal antibody will be reported separately (R. Solari *et al.*, manuscript submitted for publication). Proliferation was assessed by the incorporation of [^{3}H]thymidine over the last 16 hrs of a 72-hr incubation. The specificity of this assay was verified by using recombinant IL-2 and IL-4 (Genzyme, Boston, MA) as controls.

IFN-γ ELISA. Flat-bottomed 96-well plates (Nunc Immunotype II) were coated overnight with 50 μl/well of monoclonal antibody to IFN-γ (Biogenesis, Bournemouth, U.K.) diluted 1:500 in carbonate buffer, pH 9·6. After washing and blocking with BSA as described above, samples and standards (recombinant IFN-γ; Amersham) were incubated on the plates for 2 hr at 37°. After three washes in PBS-Tween, 5 μl of rabbit antibody to IFN-γ (Biogenesis) were added to each well at 1:200 and the incubation continued for a further hour. The washing and incubation was repeated with a 1:500 dilution of alkaline phosphatase-labelled goat antibody to rabbit immunoglobulins (Sigma). Substrate development was performed as described for the IgG and IgM ELISA. IFN-γ concentrations in test supernatants were determined from a standard curve; the assay was sensitive to a lower limit of 10U/ml IFN-γ.

Indirect immunofluorescence and flow cytometry

5×10^5 T-cell clones were incubated on ice for 30 min with 1 μg/ml of antibody to CD4, CD8 (both from Becton-Dickinson, Cowley, Oxon), CD23 (B6), CDw29 (4B4) or CD45R (H4) (all from Coulter, Luton, Beds) in MEM (Flow) containing 5% fetal calf serum (FCS) and 0·1% sodium azide. After one wash in 4 ml of the same medium, FITC-labelled goat antibody to mouse immunoglobulins (Becton-Dickinson) was added at 20 μg/ml and the incubation continued for a further 30 min on ice. After a second wash, the cells were resuspended in 1 ml of medium, and analysed on a FACS Analyser (Becton-Dickinson). Live cells were gated by forward and right-angle light scatter, and 5000 events collected in the computer.

RESULTS

Immunoglobulin production by B cells stimulated with hyper IgE clones

T-cell clones were obtained from the PBMC of a patient suffering from the hyper IgE syndrome by activation with PHA and autologous feeder cells in the presence of IL-2. Their surface phenotype was CD3$^+$, CD4$^+$, CD23$^-$, as determined by indirect immunofluorescence and flow cytometry (data not shown). Co-cultures were established with T-cell clones and B cells from three normal donors, one donor (number 4) having a history of allergy to laboratory animals. The cells were plated into tissue culture wells that had been precoated with antibody to CD3, then after 7 days of culture supernatants were harvested and assayed for IgE by a solid-phase ELISA (Table 1). The clones showed varying abilities to help IgE production by the different donors; all of the clones except H64 could stimulate at least a two-fold increase in IgE secretion by the B cells from the atopic donor (donor 4), whereas none of the clones triggered such an increase by donor 3. With the remaining donors, T-cell clones H45, H92 and H101 stimulated the best responses. Thus, although IgE helper clones can be demonstrated, it would appear that the source of the B cells is important in the generation of this response. IgM and IgG responses were also measured (data not shown). All of the clones tested were able to provide some help for the production of both isotypes regardless of the B-cell donor. However, the clones which gave the strongest IgE response also induced the highest IgM and IgG levels.

Table 1. IgE production by B cells co-cultured with T-cell clones

	IgE production by B-cell donor:			
T cell clone	1	2	3	4
—	502 (106)	201 (35)	345 (55)	345 (45)
H45	1208 (212)	560 (15)	597 (45)	1375 (141)
H64	696 (29)	225 (35)	410 (14)	675 (20)
H92	2000 (194)	423 (74)	613 (84)	1733 (255)
H101	703 (186)	657 (90)	568 (15)	1600 (250)
H114	600 (49)	340 (23)	403 (56)	817 (203)
H169	600 (30)	403 (30)	603 (81)	937 (95)
H201	473 (33)	318 (19)	415 (42)	707 (33)

B cells (E$^-$ cells) from four different donors (one to three, non-atopic; four atopic) were cultured at 5×10^5/well with an equal number of T-cell clones (in the presence of solid-phase antibody to CD3). Supernatants were harvested on Day 7 and assayed by ELISA for IgE. Values represent the mean IgE production (pg/ml) of duplicate determinations of triplicate cultures, with standard deviations from the mean shown in brackets. Underlined values represent increases in IgE secretion that are at least twice the levels secreted by B cells cultured alone.

IL-2, IL-4 and IFN-γ production by anti-CD3 triggered T-cell clones

There is now considerable evidence that the lymphokines IL-4 and IFN-γ are involved in isotype switching both *in vitro* and *in vivo*. In an attempt to correlate the ability of the T-cell clones to help IgE responses with their pattern of lymphokine secretion, activated T-cell clone supernatants were prepared by culturing for 24 hr in 48-well plates coated with antibody to CD3. The supernatants were then assayed for IL-4 and IFN-γ. IL-2 production was also measured since it has been shown to have a role in B-cell activation and differentiation (Muraguchi *et al.*, 1985; Nakagawa *et al.*, 1985; Callard *et al.*, 1986). Figure 1 shows the relative amounts of IL-2 and IFN-γ in the supernatants of the same panel of hyper IgE T-cell clones as used in the helper assays. After activation, all of the clones appeared to secrete IFN-γ. Although there was some variability in IFN-γ production, the differences did not seem to be great enough to account for the relative ability of the clones to act as IgE helper

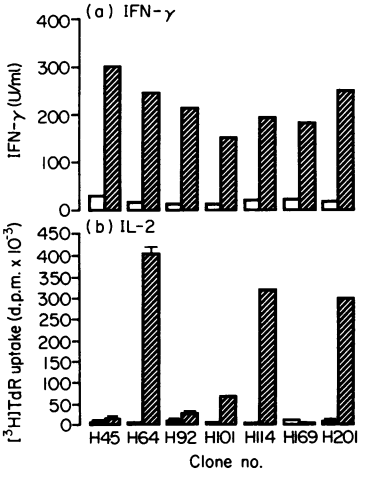

Figure 1. T-cell clones were cultured at 10^6/ml in medium alone (open bars) or in the presence of solid-phase antibody to CD3 (hatched bars). Supernatants were harvested after 24 hr and assayed for (a) IFN-γ (by ELISA) and (b) IL-2 (CTLL assay). The bars for (a) represent IFN-γ in U/ml and for (b) d.p.m. ($\times 10^{-3}$) of triplicate cultures, vertical lines represent the SD from the mean. The radiolabel incorporation by CTLL cells cultured in medium alone was 3347 (344).

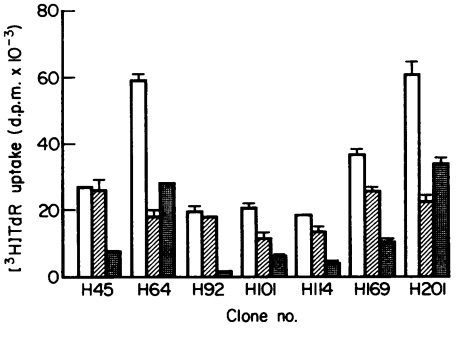

Figure 2. PHA blasts were cultured at 10^4/well in medium alone or in the presence of 24-hr culture supernatants from anti-CD3-activated T-cell clones (open bars). Rabbit antibody to IL-2 (20 μg/ml) was added to one set of replicate cultures (hatched bars) and 4B2-F9 (× 10 supernatant) to another set (black bars). Proliferation was assessed by [^{3}H]thymidine incorporation over the last 16 hr of a 72-hr culture. Bars represent the mean d.p.m. ($\times 10^{-3}$) of triplicate cultures, with SD from the mean shown by vertical lines. The radiolabel incorporation by cells cultured in medium alone was 2154 (146).

cells. The IL-2 levels however, were considerably higher with clones which were poor helpers of IgE production (H64, H114 and H201). Figure 2 shows the proliferation of PHA blasts in response to the same supernatants, either alone or in the presence of neutralizing antibodies to IL-2 or IL-4. The growth-promoting activity of culture supernatants from H45 and H92 could only be inhibited by anti-IL-4 and not anti-IL-2, showing that these clones make IL-4 and confirming that they make little IL-2. All of the other supernatants contained some growth-promoting activity that could be inhibited by both antibodies, indicating that they made both IL-2 and IL-4. The relative

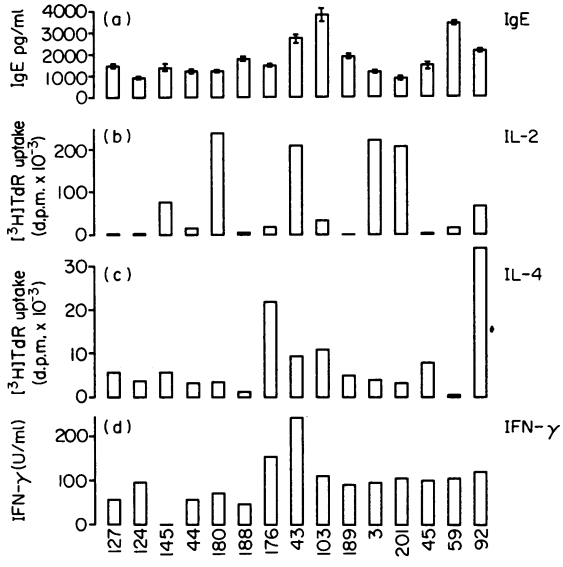

Figure 3. A larger selection of T-cell clones from the original panel were assayed for their ability to help IgE production as described in the legend of Table 1 (a). In addition culture supernatants from clones activated for 24 hr by solid-phase anti-CD3 were assayed as described previously for (b) IL-2, (c) IL-4 (shown as the proliferation of PHA blasts in the presence of neutralizing antibody to IL-2) and (d) IFN-γ.

lymphokine profiles of the clones were consistent after triggering on different occasions, although the absolute amounts were variable from one triggering to another.

To see if these observations could be confirmed, a larger selection of clones from the same panel were assayed for their ability to help IgE production and for their lymphokine secretion (Fig. 3). In this experiment, H92 was able to make a low level of IL-2, but H92 and H45 again produced higher levels of IL-4 than IL-2. Other clones such as H103 showed similar characteristics to H92, that is they produced IL-4 but low levels of IL-2 and gave good help for IgE production. However, clones such as H43 could help IgE production despite secreting high levels of both IL-2 and IFN-γ. From these results it seems clear that there is no strict correlation between a clone's ability to help IgE production and their secretion of IL-2, IL-4 or IFN-γ.

CDw29 and CD45R expression by T-cell clones

Since there was no clear correlation between the helper activity of the T-cell clones and their lymphokine secretion, other characteristics of the clones were investigated. The expression of the T-cell surface antigens CDw29 (4B4) and CD45R (2H4), antigens reported to divide $CD4^+$ T cells into two subpopulations with differing abilities to help immunoglobulin secretion (Morimoto *et al.*, 1985a, b), was examined to see if the expression of these antigens correlated with the helper function of the clones. Figure 4 illustrates the expression of these two markers on the first panel of seven clones used above. All of the clones appeared to express very little, if any, CD45R (with the possible exception of H101). However, CDw29 expression could be detected but the level of expression varied considerably depending on the T-cell clone. It was interesting to note that H45 and H92 expressed higher levels of this antigen than the

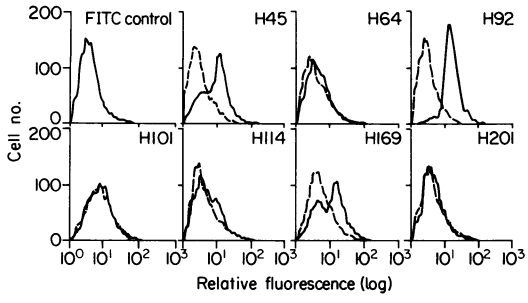

Figure 4. T-cell clones were taken 7 days after previous stimulation, then incubated overnight in fresh medium. 5×10^5 cells from each clone were stained with antibody to CDw29 (the solid line) or CD45R (the broken line). Individual panels illustrate the number of cells versus fluorescence intensity (on a logarithmic scale) for the clones, as shown.

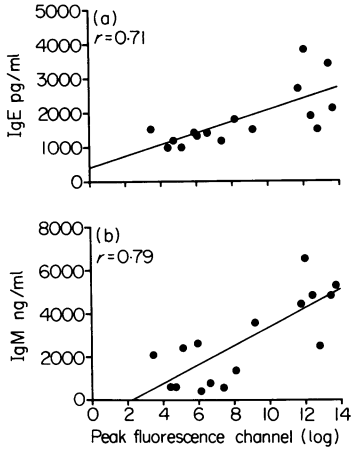

Figure 5. T-cell clones were cultured with B cells (in the presence of solid-phase antibody to CD3, as detailed in the legend to Table 1). The supernatants were harvested and assayed by ELISA for IgE and IgM. CDw29 expression of each clone was measured (as described in the legend to Fig. 4) and expressed as the peak fluorescence channel (from histograms on a logarithmic scale). Scatter diagrams were constructed of the IgE and IgM help of the clones against the CDw29 peak fluorescence channel (FITC-control peak channel = 2·3).

other clones and that they provided good help for immunoglobulin secretion. A third clone, H169, also expressed higher levels of CDw29. Although this clone did not provide such good help for IgE production, it induced significant levels of IgG and IgM (data not shown) and also made IL-4 but little IL-2 (Figs 1 and 2).

Figure 5 correlates the CDw29 expression (expressed as the peak fluorescence channel) of the larger selection of T-cell clones (Fig 3) with their ability to help IgE and IgM secretion. There was a significant correlation between the level of expression of CDw29 and the ability of the clones to help both IgE and IgM production. To examine the ability of the human T-cell clones to help IgG more closely, a selection of the culture supernatants were assayed for the levels of IgG1, IgG2, IgG3 and IgG4 secreted. Table 2 shows that the T-cell clones expressing high

Table 2. IgG subclasses secreted by B cells co-cultured with T-cell clones

		IgG subclass production by B cell			
T-cell clone	CDw29 expression	IgG1	IgG2	IgG3	IgG4
—		< 120	< 400	< 50	< 50
H43	High	3800	2000	1050	470
H92	High	8000	6400	2000	1000
H103	High	6000	4100	2000	860
H3	Low	1990	660	250	140
H64	Low	1900	920	430	180
H201	Low	3100	1500	470	320

B cells (E^- cells) from a non-atopic donor (donor 1 in Table 1) were cultured at 5×10^5 well with an equal number of T-cell clones (in the presence of solid-phase antibody to CD3). Supernatants were harvested on Day 7 and assayed by ELISA for IgG1, IgG2, IgG3, and IgG4. Values represent the mean immunoglobulin production of duplicate cultures (ng/ml). CDw29 expression by the clones was determined by flow cytometry: CDw29 high represents cells with a peak fluorescence channel > 10, CDw29 low, < 10.

levels of CDw29 were able to provide better help for the secretion of all IgG subclasses, i.e. the two groups of clones did not show preferential help for a particular IgG subclass.

DISCUSSION

The $CD4^+$ T-cell clones used in this study could be categorized as good or poor helpers according to their ability to enhance IgE secretion by B cells. The ability to help IgE responses was not isotype-specific since the same clones could also provide help for IgM and all four of the IgG subclasses. Correlating the lymphokines secreted by a small number of the clones with their ability to help immunoglobulin secretion, it appeared that those clones secreting higher levels of IL-4 and low levels of IL-2 provided the best help. However, on examination of a larger selection of clones it became clear that there was no absolute correlation between IL-2/IL-4 secretion and help for IgE production. All of the T-cell clones tested here also secreted IFN-γ, but without significant differences in amounts to account for their varying abilities to help IgE responses. In the mouse, IL-4 and IFN-γ have been shown to have reciprocal roles in the regulation of IgE responses both *in vitro* and *in vivo*; IL-4 promotes the switching of lipopolysaccharide (LPS)-activated B cells to produce IgE and IgG1, whereas IFN-γ inhibits the production of IgG3, IgG1, IgG2b and IgE whilst promoting IgG2a responses (Snapper & Paul, 1987). *In vivo*, anti-IL-4 has been shown to inhibit the IgE response to both anti-IgD and *Nippostrongilus brasiliensis* (Finkelman *et al.*, 1986, 1988). More recently, studies with human recombinant IL-4 and IFN-γ have indicated that these factors play similar roles in human IgE regulation (Yokota *et al.*, 1988). Therefore, it was surprising that the clones secreting IFN-γ were able to help IgE responses. However, recent studies by Del Prete *et al.* (1988) showed that the ability of clones secreting both IL-4 and IFN-γ to help IgE responses was dependent on the balance of the two lymphokines. In addition other lymphokines such as IL-5 have been shown to enhance IL-4-mediated IgE secretion by human

peripheral blood mononuclear cells PBMC (Pene *et al.*, 1988a, b).

Although there did not seem to be an obligate requirement for IL-4 production by IgE helper clones, preliminary studies with neutralizing antibodies to IL-4 and IFN-γ (not shown) have confirmed the observation of others (Pene *et al.*, 1988a; Del Prete *et al.*, 1988; Maggi *et al.*, 1988) that these lymphokines play important reciprocal roles in the regulation of human IgE secretion. Such antibody studies may better address the role of T-cell-derived lymphokines since there is evidence that T–B interactions may involve directed lymphokine secretion (Kupfer *et al.*, 1986; Poo, Conrad & Janeway, 1988). Thus, the levels of lymphokine measured in culture supernatants may provide a misleading picture of the amounts available to responding B cells in T–B co-culture systems. Furthermore, it has been shown that T–B interaction can enhance anti-CD3 triggered T-cell lymphokine production (Hirohata, Jelinek & Lipsky, 1988). Since the levels of production of different lymphokines vary according to the T-cell stimulus (Gauchat *et al.*, 1988) it is possible that in helper assays signals derived from T–B membrane interaction trigger the T cell to make IL-4 even though they will not with anti-CD3 alone.

Our results indicate that, unlike murine CD4$^+$ T-cell clones that have been divided into two subsets according to their lymphokine secretion (Mosmann *et al.*, 1986; Janeway *et al.*, 1988), a clear cut dichotomy between IL-4 and IL-2/IFN-γ-producing human T cells does not seem to exist. This does not appear to be due to an abnormality of the T cells from this patient with the hyper IgE syndrome since similar results have been obtained using a panel of alloreactive T-cell clones (Quint, 1988). These findings are supported by the experiments of Umetsu *et al.* (1988) using tetanus toxoid-specific and alloreactive T-cell clones derived from PBMC. In addition, a recent study using 690 PHA-driven T-cell clones derived from peripheral blood, tonsils, lymph nodes and spleens (Maggi *et al.*, 1988) showed that only 4% of CD4$^+$ T cells secreted IL-4 in the absence of IL-2 and IFN-γ, while the other clones secreted various combinations of the three lymphokines.

Although the T-cell clones used in these studies could not be divided into subsets on the basis of their lymphokine secretion, it appeared that some division could be made into good or poor helpers for immunoglobulin secretion according to their level of expression of CDw29. In most cases, high CDw29 expressors provided better help for the immunoglobulin secretion of all isotypes tested, including IgE. CDw29 was originally described as a surface antigen that could identify a subset of CD4$^+$ cells representing a true helper-inducer subset (Morimoto *et al*, 1985a). Such cells were shown to provide help for immunoglobulin synthesis by pokeweed mitogen (PWM)-stimulated B cells, whereas the reciprocal population, which is CDw29$^-$ but CD45R$^+$, was shown to induce suppression (Morimoto *et al.*, 1985a, b). More recently, it was suggested that the two populations represent different maturational stages of the same lineage, with cells expressing high levels of CDw29 (and other markers, including UCHL1, CD2, LFA-1 and LFA-3) and low CD45R being *in vivo* primed, or memory, T cells and the reciprocal population being naive or virgin T cells (Sanders *et al.*, 1988). In support of this, it has been shown that upon activation *in vitro*, the CDw29$^-$/CD45R$^+$ population lost CD45R and gained UCHL1 antigens (Akbar *et al.*, 1988). A similar division of T cells by CD45R expression has recently been reported in the mouse, although the murine high and low CD45R expressors seem to correlate with the Tinf (Th1) and Th (Th2) subsets, as defined by their lymphokine secretion (Bottomly, 1988). None of the T-cell clones used in the studies reported here expressed CD45R. This result would be expected if this marker identifies a naive T-cell population, since these clones have all been repeatedly triggered *in vitro*. Differences in lymphokine secretion, proliferative responses and ability to modulate B-cell responses by the CDw29$^+$ and CD45R$^+$ T-cell populations have been reviewed by Sanders *et al.* (1988). However, these studies used freshly isolated T cells and not T-cell clones, and no references were made to differences in the level of expression of CDw29 in CDw29$^+$/CD45R$^-$ populations.

The role of CDw29 has not yet been established, but it is a heterodimeric protein belonging to the integrin family, indicating that it may have a role in cellular adhesion (Anderson *et al.*, 1988). It is possible that high CDw29 expressors are more readily activated in the culture conditions used in the helper assays or that they form better interactions with the responding B cells, thus allowing more efficient delivery of helper signals for immunoglobulin secretion. Studies are underway to determine the effects of adding antibody to CDw29 to T–B co-cultures. In addition, high expressors of CDw29 have been shown to express higher levels of CD2, LFA-1 and LFA-3 antigens that are known to have a role in cellular adhesion and activation (Sanders *et al.*, 1988). We are therefore currently investigating the expression of these other markers on our T-cell clones and examining their role in T-cell dependent immunoglobulin secretion.

In conclusion, although human T-cell clones do not appear to divide into clear subsets as defined by their lymphokine secretion, an analysis of the level of CDw29 expression may allow a degree of division into functional subsets. T-cell clones expressing higher levels of CDw29 have a tendency to provide better help for immunoglobulin secretion, include IgE. Although the majority of clones fall clearly into this pattern, there are a few exceptions, and it may be that the expression of cell surface antigens other than those described here are of functional significance, and would further refine the definition of human helper cell populations.

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