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Generation of rat Th2-like cells *in vitro* is interleukin-4-dependent and inhibited by interferon-γ

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SUMMARY

Differentiation of naive T cells into effector cells producing T helper type 1 (Th1) and Th2 cytokines is regulated by the presence of specific cytokines in the T-cell microenvironment. The effect of interferon-γ (IFN-γ) and interleukin-4 (IL-4) on Th1- and Th2-like cell development was investigated in cultures of mixed rat spleen cells. These cells were cultured for 4 days in medium containing concanavalin A (Con A) with or without additional IL-2, IFN-γ or IL-4. The cells were then washed and their capacity to produce IL-4, IL-5 and IFN-γ determined following stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin. Freshly isolated cells stimulated with PMA and ionomycin expressed detectable levels of IL-4 and IL-5 mRNA as measured by a quantitative polymerase chain reaction (PCR) procedure and much higher levels of IFN-γ mRNA. Cells cultured with Con A for 4 days, washed, and restimulated with PMA + ionomycin were unable to express detectable levels of IL-4 and IL-5 mRNA, but produced high levels of IFN-γ mRNA. Addition of IL-4, or anti-IFN-γ antibody, to Con A-driven splenocyte cultures restored the ability of restimulated cells to express IL-4 and IL-5. $CD4^+$ T cells isolated from these cultures also showed an increased capacity to secrete IL-4 and IL-5 when anti-IFN-γ and IL-4 were present in the culture medium. When cultured for 4 days with Con-A, IL-4 and anti-IFN-γ splenocytes showed an increased capacity to proliferate in response to recombinant IL-2 and proliferated in response to IL-4 alone. IL-2 had no effect on cytokine production by cultured splenocytes. These results indicate that: (1) IL-4 is essential for the generation of Th2-like cells; (2) IFN-γ inhibits IL-4 production by mixed spleen cells and suppresses generation of IL-4 responsive T cells; (3) in mixed spleen cell cultures mitogenic stimulation favours differentiation of naive rat T cells into effector cells expressing a Th1, and not Th2, cytokine profile.

INTRODUCTION

Different subpopulations of T cells play a central role in defining the character of an immune response. In mice, two contrasting populations of $CD4^+$ T cells have been described which differ in their ability to produce certain cytokines.[1] T helper type 1 (Th1) cells produce more interferon-γ (IFN-γ), interleukin-2 (IL-2) and tumour necrosis factor-β (TNF-β), while Th2-like cells make more IL-4, IL-5, IL-6 and IL-10. Some cytokines [granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-3] are produced in similar amounts by both cell types. The development of T cells with a Th2 cytokine repertoire is believed to favour humoral immune responses and is implicated in allergic inflammatory processes, whilst Th1-like cells appear to cause delayed-type hypersensitivity reactions and favour cell-mediated immunity.[2-4] Analysis of T-cell lines has shown that although many peripheral blood T cells exhibit an unrestricted pattern of cytokine production,[5] Th1- and Th2-like cells develop during an immune response and are present at sites of inflammation.[6,7] However, the factors which direct the selective activation and differentiation of T-cell subsets are still poorly understood.

The presence of different cytokines during T-cell differentiation, as well as the nature of the antigen,[8] appear to be important in determining the functional phenotype of mature T cells. IFN-γ is reported to favour development of Th1-like cells and inhibit growth of Th2-like cells,[9-11] while addition of IL-4 to activated $CD4^+$ cell cultures induces cells that secrete high levels of Th2 cytokines[12-14] and has been shown to preferentially stimulate the growth of Th2-like cells.[11,15] The ability of IFN-γ and IL-4 to direct Th1-like and Th2-like immune responses *in vivo* has been confirmed by administration of anti-cytokine

Abbreviations: Con A, concanavalin A; dNTP, deoxynucleotide triphosphate; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; IFN-γ, interferon-γ; PMA, phorbol 12-myristate 13-acetate; PCR, polymerase chain reaction; Th, T helper.

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antibodies to mice during parasitic infections.[16,17] Recently, additional cytokines have been reported to modulate Th1/Th2-like cytokine-producing cells. Transforming growth factor-β (TGF-β) was shown to suppress the development of Th2-like effector cells,[18] and IFN-α or IL-12 to favour differentiation into Th1 cells.[19].

Although the development of different types of immunity is known to be dependent on CD4+ T cells, other cell types are also known to produce cytokines which could influence the CD4+ T-cell response. Non-T, non-B cells, probably of the mast cell/basophil lineage, produce IL-4 and other Th2 cytokines,[20] and natural killer (NK) cells produce large amounts of IFN-γ.[21] Macrophages produce IFN-α and IL-12 which activate NK cells,[19] and B cells have been shown to make IL-10 which inhibits IFN-γ production by Th1 cells.[22] Furthermore CD8+ T cells are able to produce both Th1 and Th2 cytokines[23] and can suppress the proliferation of CD4+ T cells. In this study we have used mitogen-stimulated mixed rat spleen cell cultures to analyse the effects of IL-4, IFN-γ, and IL-2 on the development of cells with the ability to produce Th1 (IFN-γ) and Th2 (IL-4 and IL-5) cytokines.

MATERIALS AND METHODS

Animals and materials

Female Hooded Lister rats (125–150 g) were purchased from Harlon-Olac Ltd (Bicester, U.K.). RPMI-1640 medium, foetal calf serum (FCS), L-glutamine, penicillin, and streptomycin were purchased from Gibco (Paisley, U.K.). Falcon tissue culture plates were supplied by Marathon Lab Supplies (London, U.K.) and microtitre plates by Nunc (Roskilde, Denmark). Recombinant human IL-2 was purchased from Euro-Cetus (Harefield, U.K.). Recombinant rat IL-4 was obtained as a supernatant from an IL-4-transfected Chinese hamster ovary (CHO) cell line (kind gift from Dr N. Barclay, MRC Cellular Immunology Unit, Oxford, U.K.). Purified recombinant IFN-γ was donated by Dr P. van der Meide (TNO, Rijswijk, The Netherlands). Rabbit anti-IFN-γ antiserum was a gift from Dr J. Tite (Wellcome Research Laboratories, Beckenham, U.K.). Dynabeads M450 sheep anti-mouse IgG were purchased from Dynal (Wirral, U.K.) and phycoerythrin-labelled OX35 monoclonal antibody (mAb) was from AMS Biotechnology (Whitney, U.K.). w3/25 anti-rat CD4 ascites fluid was from Serotec (Oxford, U.K.). ^{3}[H]Thymidine was purchased from Amersham International (Amersham, U.K.) and ionomycin from Novabiochem Ltd (Nottingham, U.K.). RNAguard, oligo-dT (12–18mer), AMV reverse transcriptase, and deoxynucleotide triphosphate (dNTP) were purchased from Pharmacia (Milton Keynes, U.K.), Perfect Match polymerase enhancer from Stratagene Ltd (Cambridge, U.K.) and Amplitaq polymerase from ILS Ltd (London, U.K.). Polymerase chain reaction (PCR) primers for rat IL-4, IL-5, IFN-γ and β-actin were purchased from British Biotechnology Ltd (Oxford, U.K.) or Molecular Medicine Unit (Kings College London, U.K.). IL-4 primers were designed from the rat IL-4 cDNA sequence[24] (kindly provided by Dr N. Barclay). Primer sequences were: IL-4: 5′ACCTTGCTGTCACCCTGTTCTGC3′ and 5′GTTGTGAGCGTGGACTCATTCACG3′, which amplify a 352 base pair (bp) fragment. IL-5: 5′TGCTTCTGTGCTTGAACGTTCTAAC3′ and 5′TTCTCTTTTTGTCCGTCAATGTATTTC3′, which amplify a 298 bp fragment and were obtained from the rat IL-5 cDNA sequence.[25] IFN-γ: 5′ACACTCATTGAAAGCCTAGAAAGTCTG3′ and 5′-ATTCTTCTTATTGGCACACTCTCTACC3′, which amplify a 432 bp fragment and were obtained from the rat IFN-γ sequence.[26] β-actin: 5′AGAAGAGCTATGAGCTGCCTGACG3′ and 5′CTTCTGCATCCTGTCAGCCTACG3′, which produce a 236 bp product and were derived from the rat β-actin sequence.[27] All other reagents were purchased from Sigma (Poole, U.K.).

Cell purification and culture

Spleens were excised from rats, pressed through wire mesh into chilled Hanks' balanced salt solution (HBSS) and passed through cotton wool in a syringe barrel. Cells were washed and 1 ml distilled water was added to lyse erythrocytes, then immediately washed twice in HBSS. Cells were resuspended and viable cell numbers determined by trypan blue exclusion. Cells were cultured at 1×10^6/ml in complete medium (RPMI adjusted to 310 mOsm + 2·2 g/l sodium carbonate, 0·3 g/l L-glutamine, 110 mg/l sodium pyruvate, 2×10^{-4}% 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin, 10% FCS) in 60 mm Petri dishes at 37°, 5% CO_2. Con A was added at 2 μg/ml, IL-2 at 20 U/ml, IFN-γ at 10 ng/ml, and IL-4-containing supernatant at a 1/500 dilution. For proliferation assays, cells were cultured at 5×10^5/ml, 200 μl/well in flat-bottomed microtitre plates for 48 hr before addition of 0·5 μCi [^{3}H]thymidine/well. Eighteen hours later cells were harvested and tritium incorporation measured using a Canberra Packard Matrix™ 96 direct beta-counter. Restimulation of cultured cells was performed after washing and resuspension in fresh medium by addition of 10 ng/ml phorbol 12-myristate 13-acetate (PMA) and 400 ng/ml ionomycin. Cells were then cultured for 6 hr at 37°, 5% CO_2.

Preparation of cDNA

Total RNA samples were prepared from cells using guanidine isothiocyanate lysis.[28] RNA pellets were washed twice with 80% ethanol, air dried and dissolved in 0·5 U/μl RNAguard, 5 mM dithiothreitol. Yields and quality of RNA were determined by spectrophotometry and analysis by agarose gel electrophoresis. Four micrograms RNA from each sample were reverse transcribed in 40 μl reverse transcriptase (RT) buffer (50 mM Tris–HCl pH 8·0, 50 mM KCl, 5 mM $MgCl_2$) containing 5 μg oligo-dT, 20 U AMV reverse transcriptase, 2·25 mM dNTP, 0·1 mg/ml BSA, 10 mM dithiothreitol, and 40 U RNAguard. Tubes were incubated at room temperature for 20 min and 30 min at 42° before storage at −20°.

PCR amplification

PCR reactions consisted of 5 μl reverse-transcribed RNA in 40 μl RT buffer containing 1·25 μM each primer, 0·3 μl 25 mM dNTP, 0·35 U Perfect Match and 1·2 U Amplitaq polymerase. Reaction mixtures were overlaid with mineral oil and transferred to a Coy Laboratory Products thermal cycler at 72°. Up to 29 PCR cycles were performed (1 min 94°, 60° for 2 min or 15 min for the first 4 cycles, 2 min 72°) and aliquots (6 μl) were taken from each sample after two or three different numbers of cycles. Aliquots of reactions were run on 3·2% agarose gels in glycine buffer stained with ethidium bromide. Photographs were

taken under ultraviolet (UV) illumination on Agfa APX25 film and the negatives were scanned on a Molecular Dynamics ImageQuant™ densitometer. Densities of marker fragments (pUC18/*Hpa*II or ϕX174/*Hin*f1 digests) were plotted against molecular weight and used to estimate yields of PCR target fragments. Copies of mRNA/μg total RNA were calculated as described previously.[29] Results were expressed as a percentage of β-actin mRNA copies.

IFN-γ secretion

IFN-γ was measured in supernatants from cells stimulated for 24 hr with PMA and ionomycin by ELISA. Nunc Maxisorb ELISA plates were coated overnight with anti-rat IFN-γ mAb (3 μg/ml in 0·1 M carbonate/bicarbonate buffer pH 9·6). Plates were washed in PBS 0·05% Tween-20 and samples were added for 2 hr at room temperature. Rabbit anti-IFN-γ antiserum was added at 1/50 in PBS 1·5% rat serum, 0·5% Tween for 1 hr, followed by alkaline phosphatase-labelled monoclonal anti-rabbit IgG (1/10,000) in the same diluent for 1 hr. Colour was developed using 0·5 mg/ml *p*-NPP substrate in 0·05 M diethanolamine buffer pH 9·8. IFN-γ levels were calculated by reference to commercial IFN-γ standards.

CD4$^+$ cell purification

CD4$^+$ cells were purified from 25 ml splenocyte cultures after 4 days by positive selection using magnetic beads. Anti-mouse IgG Dynabeads were coated overnight with w3/25 mAb at a 1/50 dilution in PBS 0·1% BSA at 4°. The beads were washed four times in PBS 0·1% BSA and 75 μl was added to the washed spleen cells which had been resuspended in 1 ml PBS 0·1% BSA. The cells were incubated at 4° on a rolling mixer for 45 min and the attached cells were collected using a magnetic particle concentrator (Dynal), washed in culture medium and directly activated with PMA and ionomycin as already described. Aliquots of the CD4$^+$ cells were retained and allowed to detach from the magnetic beads in culture medium overnight. The purity of these cells was then assessed by FACScan (Becton Dickinson, Cowley, U.K.) after staining with phycoerythrin-labelled OX35 anti-CD4 mAb.

IL-2 production

IL-2 activity in the 24 hr supernatants was assayed using the CTLL cell line (ECACC 87031904). Recombinant human IL-2 was used as a standard. The lower limit of detection was 0·2 U/ml. The specificity of this assay was confirmed by the failure of recombinant rat IL-4 to stimulate the CTLL-M cells at any concentration and the ability of anti-mouse IL-2R antibody to block proliferation by over 85%. Overall homology between rat and mouse IL-4 protein sequences is relatively low (61%)[24] and there is no reported functional homology.

Statistical analysis

All results were compared using the Mann–Whitney *U*-test. *P*-values of less than 0·05 were considered significant.

RESULTS

Effect of cytokines on generation of IL-4 and IL-5 mRNA-producing cells

Th2 cytokine expression in splenocytes was assessed using a RT-PCR assay as previously described.[29] Freshly isolated spleen cells from untreated rats were able to express IL-4 and IL-5 mRNA when stimulated with PMA (10 ng/ml) and ionomycin (400 ng/ml) for 6 hr (0·432 ± 0·0810% β-actin for IL-4, 0·0592 ± 0·0259% β-actin for IL-5). However, when spleen cells were cultured for 4 days in the presence of an optimal concentration of Con A alone (2 μg/ml), washed and restimulated with PMA and ionomycin, as described above, they were unable to express significant levels of IL-4 or IL-5 (Fig. 1). Addition of recombinant IL-4 (1/500) to the cultures restored the ability of the cells to express IL-4 and IL-5 when restimulated and the levels of mRNA then expressed were comparable to those seen with freshly isolated spleen cells. Addition of recombinant IL-2 or IFN-γ to Con A-stimulated cultures did not restore the ability of the cultured cells to express IL-4 or IL-5 and culture of cells with IL-2 or IFN-γ in addition to IL-4 and

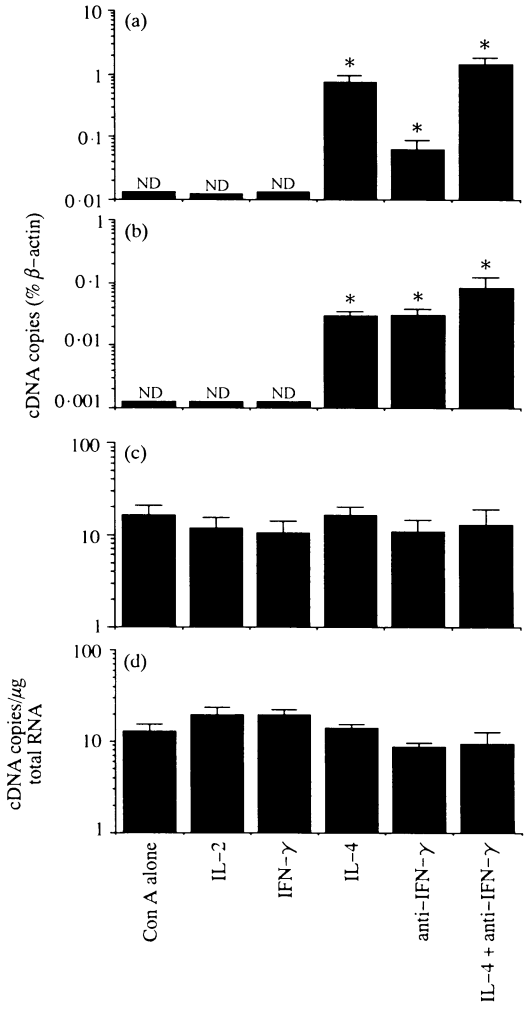

Figure 1. Expression of IL-4 (a), IL-5 (b) and IFN-γ (c) mRNA in restimulated spleen cells after culture for 4 days with Con A and the cytokines indicated. Results are expressed as a percentage of the control β-actin levels (d). Bars represent means ± SEM from four independent experiments. ND, not detectable. *Significantly different from corresponding culture with Con A alone ($P < 0·05$, Mann–Whitney *U*-test). Levels of expression in freshly isolated spleen cells were: 0·432 ± 0·0810% β-actin for IL-4, 0·0592 ± 0·0259% β-actin for IL-5, and 5·28 ± 1·64% β-actin for IFN-γ.

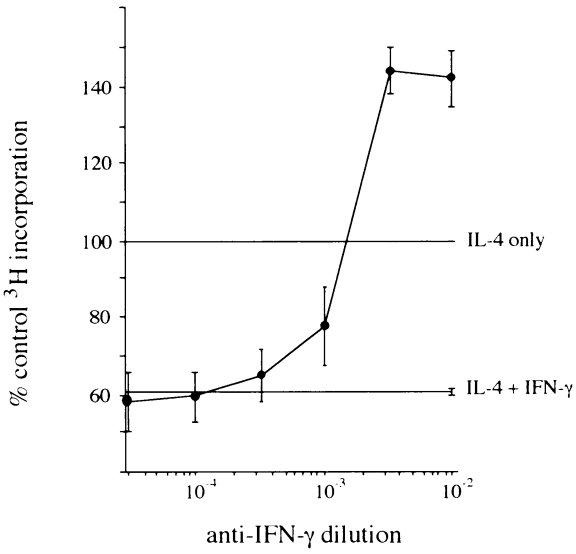

Figure 2. Anti-IFN-γ antiserum reverses the inhibition of IL-4-induced proliferation caused by IFN-γ. Spleen cells were cultured with PMA and IL-4 with recombinant IFN-γ for 2 days before addition of [^{3}H]thymidine for 18 hr. Data represent means ± SEM from three independent experiments.

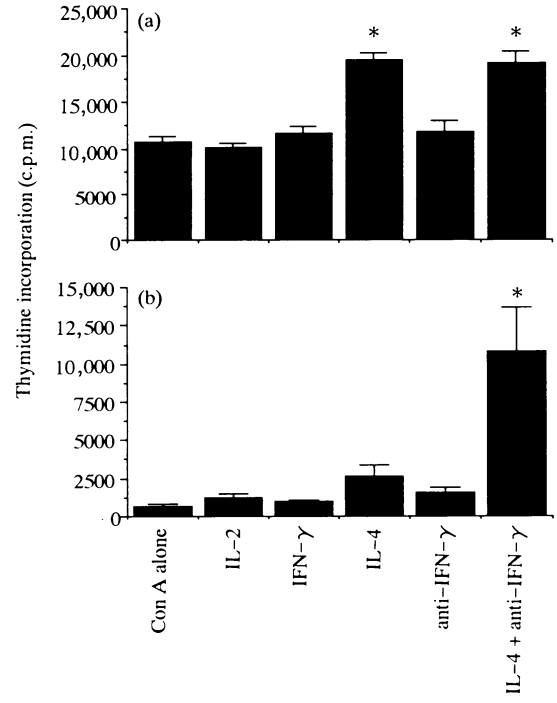

Figure 4. Proliferation of spleen cells in response to IL-2 (a) and IL-4 (b) after expansion with Con A and the cytokines indicated. Washed cells were cultured with IL-2 (50 U/ml) or IL-4 (1/500) for 2 days before pulsing with [^{3}H]thymidine for 18 hr.

Con A did not alter the levels of IL-4 and IL-5 mRNA obtained after restimulation (results not shown, $P > 0{\cdot}05$, Mann–Whitney U-test). The concentrations of IL-2 and IL-4 used in the cultures were those which induced optimal proliferation of splenocytes in combination with PMA (10 ng/ml) (results not shown). The concentration of IFN-γ used was sufficient to inhibit PMA + IL-4-induced proliferation by ~50% (see below).

The role of IFN-γ in Th1/Th2-like differentiation was further investigated by blocking endogenous IFN-γ activity in the primary cultures with anti-IFN-γ antiserum. The concentration of antiserum used (1/100), was sufficient to reverse the inhibition of proliferation caused by addition of IFN-γ to PMA + IL-4-activated spleen cells (Fig. 2), and the enhancement of proliferation above control levels caused by anti-IFN-γ suggests that endogenous IFN-γ activity was also blocked.

Addition of anti-IFN-γ to primary cultures of Con A-stimulated spleen cells significantly enhanced the ability of the cells to express the IL-4 and IL-5 genes when restimulated ($P < 0{\cdot}05$, Mann–Whitney U-test; Fig. 1). Anti-IFN-γ and IL-4 induced the highest levels of IL-4 and IL-5 mRNA expression. Levels of IL-4 expression in these cells (1·28 ± 0·322% β-actin) were significantly greater than those seen in fresh spleen cells (0·588 ± 0·0735% β-actin, $P < 0{\cdot}05$, Mann–Whitney U-test). The levels of β-actin expression showed no significant differences between cultures.

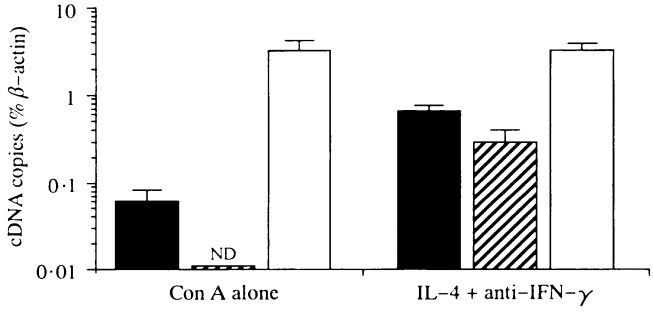

Figure 3. Production of IL-4 (■), IL-5 (▨) and IFN-γ (□) mRNA from purified CD4$^+$ cells after culture for 4 days in the presence of Con A + IL-4 and anti-IFN-γ or Con A alone. Cells were purified from splenocyte cultures using anti-CD4-coated magnetic beads and restimulated with PMA and ionomycin. Bars represent means ± SEM from three independent experiments.

Effect of cytokines on the generation of IFN-γ mRNA-producing and IFN-α-secreting cells

In vitro stimulation of spleen cells with Con A had little effect on the capacity of the cells to express the IFN-γ gene (Fig. 1). Addition of IL-4, IL-2, IFN-γ, or blocking of endogenous IFN-γ activity with anti-IFN-γ antibody had no effect on the ability of the cells to produce IFN-γ mRNA. In the same experiment no effect of these cytokines on IFN-γ secretion was seen (results not shown).

Cytokine expression in purified CD4$^+$ cells

In order to confirm that the effects of cytokines on splenocyte differentiation were attributable to CD4$^+$ T cells, further experiments were performed in which CD4$^+$ cells were purified from Con A-stimulated cultures of splenocytes, or from cultures stimulated with Con A, IL-4 and anti-IFN-γ. The CD4$^+$ T cells were >95% CD4$^+$ as assessed by FACScan. Expression of IL-4, IL-5 and IFN-γ mRNA in purified CD4$^+$ cells was similar to that observed in mixed splenocytes (Fig. 3), although IL-4 expression was now detectable in CD4$^+$ cells after culture in

Con A alone. The ability of IL-4 and anti-IFN-γ treatment to enhance subsequent IL-4 and IL-5, but not IFN-γ expression was also observed with the purified CD4$^+$ cells.

Proliferative responses of cells before and after primary culture

To determine whether the effects of cytokines on Th2-like cell generation could be accounted for by differential effects on cell growth in the primary cultures, duplicate cultures were pulsed with [^{3}H]thymidine for 18 hr and tritium incorporation measured. Addition of cytokines to the cultures had no additional effect on the proliferation of Con A-stimulated splenocytes (results not shown).

Since Th2 cells are known to be more responsive to IL-4 as a growth factor than Th1 cells, the ability of spleen cells to proliferate in response to IL-4, and IL-2, after primary culture with Con A and cytokines was studied. Washed cells from primary cultures were grown at 2×10^5/ml with 50 U/ml IL-2 or IL-4 (1/500) for 2 days and tritium incorporation assessed. The results (Fig. 4) show that cells cultured in Con A alone were unable to proliferate when restimulated with IL-4, but were responsive to IL-2. In contrast, cells cultured with both IL-4 and anti-IFN-γ were responsive to IL-4, and had slightly enhanced responsiveness to IL-2 regardless of anti-IFN-γ ($P < 0{\cdot}05$; Mann–Whitney U-test). Proliferation of cells restimulated with PMA and ionomycin was not significantly affected by addition of cytokines to the primary culture (results not shown).

DISCUSSION

In vitro studies with murine lymphocytes have shown that the cytokines IL-4 and IFN-γ are able to regulate the differentiation of naive T cells into those which secrete a Th1 or Th2 pattern of cytokines.[30] Freshly isolated murine lymphocytes were able to produce limited amounts of Th2 cytokines, but following culture in the presence of Con A or following ligation of the T-cell receptor with anti-CD3 antibodies, a population of cells able to secrete high levels of Th2 cytokines was produced. This process was enhanced by IL-4,[12,31,32] while addition of IFN-γ inhibited the development of these Th2-like cells.[32,33] We have investigated the effects of recombinant rat IL-4, IFN-γ and IL-2 on T-cell subset differentiation in cultures of rat splenocytes. A mixed cell population rather than purified T cells was used for these studies as we believe that such cells may more closely reflect what happens *in vivo*.

In the absence of IL-4, cultured spleen cells lost the capacity to produce IL-4 and IL-5. This is in contrast to the data obtained in mice where addition of Con A alone or T-cell receptor ligation appear to be a sufficient stimulus for the generation of Th2-like cells.[34,35] It seems likely that this difference is at least partly due to endogenously secreted IFN-γ as the capacity to produce IL-4 and IL-5 was restored if the cells were cultured in the presence of anti-IFN-γ. Addition of IL-4 and anti-IFN-γ permitted an expansion of cells with a Th2 cytokine profile confirming the observations made in mice on the effects of IL-4 and IFN-γ on Th2-like cell development.[31–33] The down-regulation of Th2-like cells we observed in our cultures was partly due to the balance of endogenous cytokines produced, as addition of IL-4 or anti-IFN-γ restored the ability of cells to synthesize IL-4 and IL-5. Expansion of Th2-like cells may also have been limited by the presence of other cytokines (e.g. TGF-β), or CD8$^+$ cells. Since the larger, Con A-stimulated cells yielded much higher levels of RNA than fresh splenocytes, production of cytokines/cell would have been greater in these cells but correction for total RNA and β-actin levels removes this effect from our results. Since cells cultured in Con A alone expressed high levels of IFN-γ mRNA, it is likely that many cells were already committed to a Th1 phenotype. The levels of IL-4 and IL-5 mRNA detected in fresh spleen cells after stimulation suggest that significant numbers of Th2-like cells are present *in vivo*, but these do not persist in culture. This may reflect the *in vivo* observation that Th2-like cells are short-lived.[36]

While normal rat splenocytes are unable to proliferate in response to IL-4 alone, cells cultured in the presence of both IL-4 and anti-IFN-γ showed substantial levels of proliferation when cultured with recombinant IL-4. This shows that Th2-like effector cells were generated in these cultures, since Th2 clones are highly responsive to IL-4. Cells cultured in IL-4 also had slightly increased responsiveness to IL-2. The rapid expansion in Th2 cells observed during immune responses[31,36] may, therefore, be assisted by the ability of developing effector cells to grow rapidly in response to autocrine IL-4 and IL-2, and to suppress IFN-γ production.

Addition of cytokines, or blocking of endogenous IFN-γ activity with anti-IFN-γ antibody, had no effect on the ability of the cells to make IFN-γ. The generation of Th1-like cells in this culture system does not appear to be dependent on IFN-γ. IL-4 has been reported to suppress the generation of IFN-γ-producing CD4 cells.[12] In some experiments IL-4 suppressed IFN-γ secretion while in others it had little effect and there did not appear to be any effect of exogenous IL-4 on IFN-γ mRNA expression (unpublished observation). It is therefore possible that IFN-γ production is regulated by post-transcriptional events. The very high levels of IFN-γ mRNA expression we observed in both fresh and cultured cells compared to IL-4 and IL-5 indicates that Th2 cytokine production is more tightly regulated than IFN-γ in the rat. Since generation of Th2 effector cells in our system was dependent on exogenous IL-4, it seems likely that development of Th2 cells *in vivo* requires the presence of accessory cell types which produce this cytokine.

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