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Restricted production of interleukin 4 by activated human T cells

(interferon γ/interleukin 2/lymphokines/helper T cells/*in situ* hybridization)

DAVID B. LEWIS*†, KATHRYN S. PRICKETT‡, ALF LARSEN‡, KENNETH GRABSTEIN‡, MICHAEL WEAVER*, AND CHRISTOPHER B. WILSON*

*Division of Infectious Disease, Department of Pediatrics, University of Washington, and Children's Hospital and Medical Center, 4800 Sand Point Way, N.E., Seattle, WA 98105; and ‡Immunex Corporation, 51 University Street, Seattle, WA 98101

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ABSTRACT **Interleukin 4 (IL-4) is secreted by activated T cells and pleiotropically modulates both B- and T-lymphocyte function. In murine helper ($CD4^+$) T-cell clones IL-4 production appears to be regulated independently of interferon γ and interleukin 2. To determine whether production of these lymphokines is also differentially regulated in uncloned human T cells, we studied lymphokine production by normal human peripheral T cells and T-cell subsets after *in vitro* polyclonal activation. After maximal induction of lymphokine expression, IL-4 mRNA was detectable in <5% of $CD4^+$ and 1–2% of unfractionated T cells, whereas ≈33% and 60% of $CD4^+$ cells expressed detectable mRNA for interferon γ and interleukin 2, respectively. This finding correlated with dramatically lower production of IL-4 mRNA and protein than of interferon γ and interleukin 2 by peripheral blood and tonsillar T cells. The helper-inducer ($CD4^+$ $CD45R^-$) T-cell subset, which significantly enhances *in vitro* immunoglobulin production, accounted for the preponderance of IL-4 mRNA accumulation and protein production by $CD4^+$ T cells; nevertheless, cells with detectable IL-4 mRNA constituted <10% of the $CD4^+$ $CD45R^-$ subset. Limitation of IL-4 production to a comparatively small population of normal human T cells could selectively regulate the effects of this lymphokine in T-cell-mediated immune responses; such selective regulation may be a fundamental mechanism for restricting the potentially pleiotropic effects of certain lymphokines to appropriate responder cells.**

Interleukin 4 (IL-4) is a T-cell lymphokine that modulates lymphocyte function by promoting B-cell growth, regulating immunoglobulin isotype expression, and promoting T-cell growth and cytotoxicity (1–4). Interferon γ (IFN-γ) (5, 6) and, to a lesser extent, interleukin 2 (IL-2) (6, 7), which are primarily produced by activated T cells, also have pleiotropic effects on lymphoid cells. In certain cases all three lymphokines may have similar or synergistic activities, such as the enhancement of T-cell-mediated cytotoxicity (4, 8), whereas in other situations, such as the regulation of immunoglobulin isotype expression by IL-4 and IFN-γ, differential or antagonistic effects have been demonstrated (2, 9, 10).

These *in vitro* findings raise the question as to how lymphokine production by activated T cells is regulated *in vivo* to result in an integrated effective immune response. One mechanism to facilitate distinct T-cell effector functions would be to limit the production of a lymphokine to a particular subtype of T cells. Such segregation of $CD4^+$ T-cell lymphokine production has been proposed by Mosmann and co-workers (11, 12) from the observation that most murine $CD4^+$ clones produce either IL-4 or IFN-γ and IL-2 in a mutually exclusive fashion. However, most human $CD4^+$ T-cell clones do not appear to conform to this pattern (9, 13), and there is evidence that the patterns of lymphokine production obtained in clones may be influenced by the conditions used during the cloning process (14). In addition, clone analysis does not inform about the relative frequency of primary T cells producing any particular lymphokine. Although examining lymphokine production by the progeny of single activated T cells obtained after limiting dilution technique can yield an approximate estimate of precursor frequency, the potential remains for changes in pattern of lymphokine production during the multiple replications of cells required by this technique. As an alternative to these approaches, we used mRNA hybridization techniques and specific RIAs to compare IL-4, IFN-γ, and IL-2 production by uncloned peripheral human T cells and T-cells subsets. We found a strikingly lower frequency of IL-4-producing cells compared with those producing IFN-γ or IL-2 in unfractionated as well as $CD4^+$ and $CD8^+$ T cells.

MATERIALS AND METHODS

Cells. Whole mononuclear (WM) cells were isolated from the peripheral blood (PB) of adult volunteers or minced tonsil tissue by centrifugation over Ficoll/Hypaque gradients, and T cells were purified from WM cells by treatment with T-cell Lymphokwik, a proprietary mixture of monoclonal antibodies (mAb) and complement (One Lambda, Los Angeles), as described (15). $CD4^+$ and $CD8^+$ T-cell subsets were purified by treating T cells with complement and either anti-CD8 (OKT8) (16) or anti-$CD4^+$ (66.1) (17) mAb, respectively; the anti-CD16 mAb FC-1 (18) was included in these treatments to deplete residual natural killer cells. $CD4^+$ T cells were further fractionated into $CD45R^+$ and $CD45R^-$ populations by incubation with mAb UCHL-1 (19) or 3AC5 (20), respectively, followed by negative selection with indirect panning using plastic Petri dishes coated with goat anti-mouse IgG (Tago) (21). The purity of all T-cell subsets was >95% as determined by immunofluorescent flow cytometry after indirect staining with the appropriate mAb.

Cell Activation. Cells were incubated at 5×10^6 per ml in either RPMI 1640 medium with 5% (vol/vol) human AB serum for RNA isolation and *in situ* hybridization experiments, or serum-free AIM-V medium (GIBCO) for assay of IL-4 and IFN-γ protein in cell supernatants. Both media were supplemented with L-glutamine (2 mM), penicillin G (50 units/ml), and streptomycin (50 μg/ml). T cells were activated using either ionomycin (0.5 μm; Calbiochem), Con A (25 μg/ml; Pharmacia), or OKT3 mAb (16) [1:25 (vol/vol) of sterile azide-free ascites] in combination with phorbol 12-myristate 13-acetate (PMA) (50 ng/ml; Sigma). WM cells were incubated with Con A (25 μg/ml) alone.

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Abbreviations: EF, elongation factor 1-α; IFN-γ, interferon γ; IL-2, interleukin 2; IL-4, interleukin 4; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate; PB, peripheral blood; WM, whole mononuclear.

†To whom reprint requests should be addressed.

IL-4 and IFN-γ Assay. Supernatants from WM-cell, T-cell, or T-cell subset cultures were concentrated 20- to 50-fold using a Centricon-10 apparatus (Amicon) that had been preincubated with AIM-V medium/0.2% bovine serum albumin to reduce protein absorption. Recovery of samples spiked with recombinant human IL-4 or recombinant human IFN-γ and concentrated was 70–80%. The IL-4 content of concentrated supernatants was determined by RIA. Samples or known standards were serially diluted and incubated overnight with unglycosylated recombinant human IL-4, polyclonal rabbit anti-human IL-4 serum, and protein A-Sepharose (Sigma). The antiserum was prepared by injecting unglycosylated recombinant human IL-4 subcutaneously; for primary immunization the protein was emulsified in Freund's complete adjuvant and for booster immunizations, in Freund's incomplete adjuvant. After repeated centrifugation and washing of the pellet, ^{125}I content was determined, and the sample concentration of IL-4 was interpolated from the results of the standard curve. Recombinant human IFN-γ; interleukins 1α and 1β; IL-2; interleukins 5, 6, and 7; granulocyte colony-stimulating factor, granulocyte/macrophage colony-stimulating factor, and colony-stimulating factor type 1 were all unreactive in this assay at concentrations of 1 μg/ml. IFN-γ content was similarly determined using a commercially available RIA kit (Centocor, Malvern, PA) (22). The IL-4 and IFN-γ molarities were calculated for cell culture supernatants before their concentration assuming 100% recovery.

RNA Blot Analysis. Total cellular and polyribosomal RNA were isolated by the guanidinium thiocyanate/cesium chloride method (23); polyribosomes were isolated by magnesium precipitation of 2% (vol/vol) Triton X-100 lysates as described (24) in the presence of vanadyl ribonucleosides (20 mM) (Bethesda Research Laboratories). Five micrograms of total cellular RNA or 1 μg of polyribosomal RNA were analyzed in each lane by electrophoresis in 2.2 M formaldehyde/1% agarose gels and blotting as described (25). ^{32}P-labeled RNA probes were synthesized from human IFN-γ, IL-4, and IL-2 cDNAs subcloned into transcription vectors (25). The human elongation factor-1α (EF) cDNA was ^{32}P-labeled by the random hexamer primer method (26). EF mRNA, which comprises ≈0.5% of mRNA in most mammalian cells (C. Beals and R. Perlmutter, personal communication), served as a positive control for uniform loading and blotting of RNA samples. cDNAs used for probe synthesis were all full length (4, 27) [IL-4, 0.6 kilobase (kb); IL-2, 0.9 kb; and EF, 1.9 kb] except for the IFN-γ cDNA, which consisted of a 0.4-kb internal fragment (25). Hybridization and washing of filters was as described (25) except that the EF hybridization was at 42°C.

***In Situ* Hybridization.** T cells or T-cell subsets were activated with ionomycin and PMA for 6 hr, cytocentrifuged, fixed in 4% paraformaldehyde (wt/vol)/phosphate-buffered saline for 1 min and stored in 70% ethanol at 4°C. High-specific-activity ^{35}S-labeled single-stranded antisense or sense RNA probes were transcribed from the human IL-4, IFN-γ, and IL-2 cDNAs described above. Hybridization, washing, emulsion coating, and development after 4–35 days of exposure were done as described (28). Slides were counterstained with Dif-Quik (Dade Diagnostics, Aguada, PR), permanently mounted, and coded. For each slide, the number of grains over 400 individual cells was determined by counting 100 cells in each of four randomly selected fields that were free of obvious artifacts. For each mRNA, cells hybridized with an antisense probe were considered positive when the number of grains per cell was greater than the grain count of 99% of cells hybridized with a control sense probe.

RESULTS

IL-4 and IFN-γ Protein Production. To compare IL-4 and IFN-γ production by activated T cells, IL-4 and IFN-γ protein content in ionomycin and PMA-treated T-cell culture supernatants were quantitated by specific RIA. The relative insensitivity of the IL-4 RIA (lower limit of detection of 1–2 ng/ml of recombinant human IL-4) required the concentration of supernatants before assay. To facilitate this concentration cells were cultured in a serum-free medium that supported cell proliferation, as determined by [^3H]thymidine incorporation, and expression of IL-4, IFN-γ, and IL-2 mRNA as effectively as serum-containing medium (data not shown). The molarity of IFN-γ protein was more than one hundred times greater than that of IL-4 protein in T-cell or WM-cell culture supernatants after 24-hr incubation with ionomycin and PMA (Table 1, Exp. 1 and 2). A similar ratio of IFN-γ to IL-4 protein concentration was measured after activation of T cells or WM cells with several other stimuli, although these stimuli were clearly less efficient than ionomycin and PMA in inducing secretion of these lymphokines.

IL-4, IFN-γ, and IL-2 mRNA Expression by Activated T Cells. To determine whether the accumulation of IL-4 mRNA in activated T cells paralleled the relatively low levels of secreted IL-4 protein measured in culture supernatants, T cells were analyzed for IL-4, IFN-γ, and IL-2 transcripts after either ionomycin and PMA or Con A and PMA treatment. No IL-4, IFN-γ, or IL-2 transcripts were detectable in unactivated T cells (Fig. 1*A*). After activation with either stimulus, the accumulation of all three lymphokine mRNAs peaked at ≈6 hr and subsequently declined (Fig. 1*A*). Strikingly, the detection of IL-4 mRNA required a 20- to 50-fold longer autoradiographic exposure than was necessary for the detection of IFN-γ or IL-2 transcripts (Fig. 1). Because probes for all three lymphokine mRNAs were synthesized from cDNAs of similar size and G+C content and were used under identical conditions of hybridization and washing, IL-4 mRNA appeared markedly less abundant than IFN-γ or IL-2 mRNA in activated T cells.

The amount of IL-4 mRNA detected in tonsillar T cells was similar or lower than that of identically treated PB T cells (Fig. 1*D*), suggesting that IL-4 production was reduced compared with IFN-γ or IL-2 production in both circulating and lymphoid tissue-derived T cells. IL-4 mRNA levels also appeared reduced compared with IFN-γ or IL-2 mRNA after activation of T cells with anti-CD3 (OKT3) mAb and PMA or WM cells with Con A, although in agreement with the protein assays, these conditions resulted in much lower amounts of all three lymphokine mRNAs than either ionomycin or Con A in combination with PMA (data not shown). Consistent with the earlier finding of low amounts of secreted IL-4 protein in T-cell culture supernatants, IL-4 transcripts associated with polyribosomes, and presumably in the process of

Table 1. Concentrations of IL-4 and IFN-γ protein in supernatants of activated PB T-cell and WM-cell cultures after 24-hr incubation

Exp.	Cell type: Cell	Cell type: Donor	Activation	IL-4, pM	IFN-γ, pM
1	T cells	A	Ionomycin + PMA	14.6	1762
	T cells	B	Ionomycin + PMA	34.6	4164
	T cells	C	Ionomycin + PMA	26.8	4766
2	T cells	D	Ionomycin + PMA	31.0	5510
	T cells	D	Con A + PMA	8.0	1825
	T cells	D	OKT3 + PMA	<3.5	445
	WM cells	D	Con A	<3.5	505
3	$CD4^+$ T cells	E	Ionomycin + PMA	28.0	3340
	$CD4^+$ $CD45R^+$	E	Ionomycin + PMA	<3.5	335
	$CD4^+$ $CD45R^-$	E	Ionomycin + PMA	57.5	4895

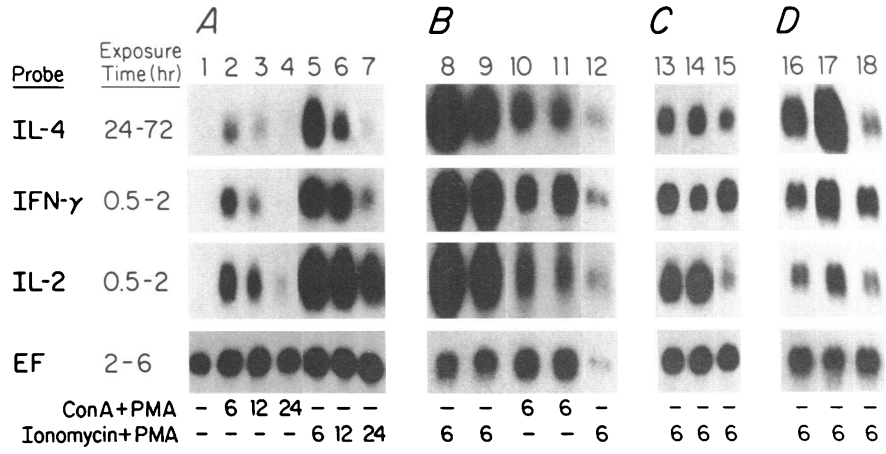

FIG. 1. RNA blot analysis of activated peripheral T cells. (*A–D*) Individual RNA blots sequentially probed for IL-4, IFN-γ, IL-2, and EF mRNA. (*A*) Kinetics of mRNA accumulation in PB T cells from a single donor after no stimulation (lane 1); activation with Con A and PMA for 6, 12, and 24 hr (lanes 2–4, respectively); or ionomycin and PMA for 6, 12, and 24 hr (lanes 5–7, respectively). (*B*) PB T-cell total RNA from two donors, 1 (lanes 8 and 10) or 2 (lanes 9 and 11), and PB T-cell polyribosomal RNA (lane 12) after T-cell activation with ionomycin and PMA (lanes 8, 9, and 12) or Con A and PMA (lanes 10 and 11) for 6 hr. (*C* and *D*) mRNA accumulation after 6 hr or activation with ionomycin and PMA in unfractionated (lane 13), $CD4^+$ (lane 14), and $CD8^+$ (lane 15) PB T cells from one donor, unfractionated $CD4^+$ (lane 16) and $CD4^+$ $CD45R^-$ PB T cells (lane 17) from another donor, and tonsillar T cells (lane 18) from a 10-yr-old girl with recurrent tonsillitis.

undergoing translation, were relatively rare compared with IFN-γ and IL-2 mRNA (Fig. 1*B*). Taken together, these results suggest that the production of IL-4 protein is largely regulated pretranslationally and that the apparently low amount of T-cell IL-4 mRNA which accumulated after T-cell activation accounted for the relatively low levels of IL-4 compared with IFN-γ protein measured in cell culture supernatants.

IL-4 mRNA Expression and Protein Production by T-Cell Subsets. To examine activated T cells for heterogeneity in IL-4 production, purified $CD4^+$ and $CD8^+$ T cells were compared for IL-4, IFN-γ, and IL-2 transcript accumulation after ionomycin and PMA treatment for 6 hr; ionomycin and PMA were used because they consistently induced the highest levels of lymphokine mRNA (Fig. 1*B*) and protein (Table 1), and this allowed a better assessment of the maximal potential of T cells to produce these proteins. $CD4^+$ T cells were enriched in IL-4 and IL-2 mRNA compared with $CD8^+$ and unfractionated T cells (Fig. 1*C*). In contrast, IFN-γ mRNA was more abundant in $CD8^+$ than in $CD4^+$ and unfractionated T cells (Fig. 1*C*). Based on densitometry of RNA blots of activated CD4 and CD8 T cells isolated from three different healthy adults (data not shown), IL-4 mRNA was 1.2–2.5 times more abundant in $CD4^+$ than $CD8^+$ T cells after ionomycin and PMA activation for 6 hr.

Circulating human $CD4^+$ T cells can be further separated into two mutually exclusive subsets, approximately equal in number, based on surface expression of different forms of the leukocyte common antigen. A 200- to 220-kDa form of the leukocyte common antigen expressed by $CD4^+$ T cells has been designated CD45R (29) and is recognized by the mAb 2H4 or 3AC5 (20), whereas the $CD4^+$ $CD45R^-$ subset expresses the 180-kDa form of the leukocyte common antigen recognized by mAb UCHL-1 (19, 30). When purified $CD45R^+$ and $CD45R^-$ $CD4^+$ PB T cells were activated with ionomycin and PMA, the $CD45R^-$ subset was markedly enriched for IL-4 transcripts and IL-4 protein production compared with unfractionated $CD4^+$ T cells (Table 1, Fig. 1*D*), whereas IL-4 protein production by $CD45R^+$ cells was below the limit of detectability (Table 1). The $CD45R^-$ subset was also consistently enriched for IFN-γ mRNA and protein production (Table 1, Fig. 1*D*). Although higher levels of IL-2 mRNA were also detected in $CD45R^-$ cells compared with unfractionated $CD4^+$ T cells in the experiment shown here (Fig. 1*D*), this enrichment, unlike that for IL-4 or IFN-γ, was inconsistent when T cells from several different donors were analyzed (data not shown). The differences between $CD45R^-$ and $CD45R^+$ cells did not appear to be an artifact of the separation procedures because treatment of $CD4^+$ T cells with mAb UCHL-1 or 3AC5 had no effect on the accumulation of IL-4 or IFN-γ mRNA after ionomycin and PMA activation (data not shown).

Frequency of IL-4-, IFN-γ-, and IL-2-Producing Cells. The relatively low levels of IL-4 mRNA accumulation and protein production by T cells and T-cell subsets suggested that either IL-4-producing T cells expressed IL-4 transcripts at low levels or that IL-4 production was restricted to a small number of cells compared with IFN-γ and IL-2. To distinguish between these possibilities, activated T cells were analyzed for lymphokine mRNA accumulation with *in situ* hybridization, the data from which are summarized in Table 2. To maximize sensitivity for the detection of lymphokine mRNA, ionomycin and PMA were used for cell activation in these studies; more physiologic stimuli such as anti-CD3 mAb and PMA resulted in levels of IL-4, IFN-γ, and IL-2 transcripts that were <10% of those observed in ionomycin- and PMA-activated T cells based on densitometry of RNA blot autoradiographs (data not shown). IL-4 mRNA expression was detected in a discrete population comprising only 1–2% of unfractionated T cells (Fig. 2 *A* and *C*), 3–4% of $CD4^+$ T cells (Fig. 2 *E* and G), and 8% of the $CD4^+$ $CD45R^-$ subset. The percentage of $CD4^+$ T cells with detectable IL-4 transcripts only increased slightly after autoradiographic exposure for 5 weeks. In contrast, IFN-γ (Fig. 2 *B* and *D*) and IL-2 transcripts were detected in ≈50% of unfractionated peripheral T cells. For all three lymphokine mRNAs, unstimulated T cells were always negative—the distribution of grains in unstimulated T cells hybridized with antisense-strand probe was similar to that of activated T cells after hybridization with a sense-strand probe (data not shown).

The observed low percentage of cells containing IL-4 transcripts was unlikely to be explained by a reduced sensitivity for the detection of IL-4 relative to IFN-γ or IL-2 mRNA. The percentage of cells positive for IL-4, IFN-γ, and IL-2 mRNA were proportional to their relative abundance as detected by RNA blotting (Fig. 1) and to the molar concentration of IL-4 and IFN-γ protein in cell-culture supernatants (Table 1). In addition, the majority of IL-4 mRNA-positive cells had many more grains per cell compared with cells hybridized with an IL-4 sense-strand control probe (Fig. 2*C*).

Table 2. IL-4, IFN-γ, and IL-2 mRNA expression in activated peripheral T cells and T-cell subsets

Cell type	Exposure time, days			Cells positive for mRNA, %		
	IL-4	IFN-γ	IL-2	IL-4	IFN-γ	IL-2
T cells (donor A)	14	7	7	2	42	58
T cells (donor B)	9	9	9	2	39	48
T cells (donor C)	11	4	4	1	42	39
$CD4^+$ T cells (donor C)	11	4	4	4	36	62
$CD8^+$ T cells (donor C)	11	4	4	<1	76	22
$CD4^+$ T cells (donor D)	14	7	7	3	34	57
$CD4^+$ T cells (donor D)	35	ND	ND	4	ND	ND
$CD8^+$ T cells (donor D)	14	7	7	1	58	16
$CD4^+$ T cells (donor E)	11	11	ND	3	36	ND
$CD4^+$ $CD45R^-$ cells (donor E)	11	11	ND	8	51	ND

ND, not done.

Finally, when $CD4^+$ T cells were hybridized and then exposed so that the highest grain count of cells with detectable IL-4 mRNA exceeded the maximum grain count of cells with detectable IFN-γ mRNA, the number of positive cells for IFN-γ mRNA (Fig. 2 *F* and *H*) still exceeded that for IL-4 mRNA (Fig. 2 *E* and *G*) by 7-fold.

DISCUSSION

We demonstrated that the accumulation of IL-4 mRNA and the secretion of IL-4 protein by activated human peripheral T cells were markedly lower than for IFN-γ or IL-2. This difference was due to the low frequency of cells expressing IL-4 mRNA compared with those containing IFN-γ or IL-2 transcripts. We also found that the overall rate of IL-4, IFN-γ, and IL-2 gene transcription in activated T cells is proportional to accumulated mRNA and to the respective frequency of cells expression cognate mRNAs (D.B.L. and C.B.W., unpublished work). IL-4, IFN-γ, and IL-2 were coordinately expressed after T-cell activation, and the relative efficiency of different activation stimuli in inducing their production was identical for these three lymphokine genes; this coordination suggests that they share, in part, initial signal transduction pathways (31, 32). Thus the mechanism responsible for their differential expression presumably regulates transcription either at the level of the gene or at a more distal selective point in signal transduction.

Fractionation experiments revealed that most IL-4-producing T cells had a $CD4^+$ $CD45R^-$ (helper/inducer) surface phenotype, a subset previously reported to augment B-cell immunoglobulin production *in vitro* (33). These findings are consistent with the proposal that IL-4 is a major B-cell helper factor (1, 34); the higher percentage of IFN-γ-producing cells in the $CD4^+$ $CD45R^-$ subset compared with unfractionated $CD4^+$ cells might also contribute to helper/inducer activity because IFN-γ also can enhance immunoglobulin production in polyclonally activated B cells (35). The expression of IL-4 mRNA by activated $CD8^+$ T cells is also interesting in light of recent observations that IL-4 can

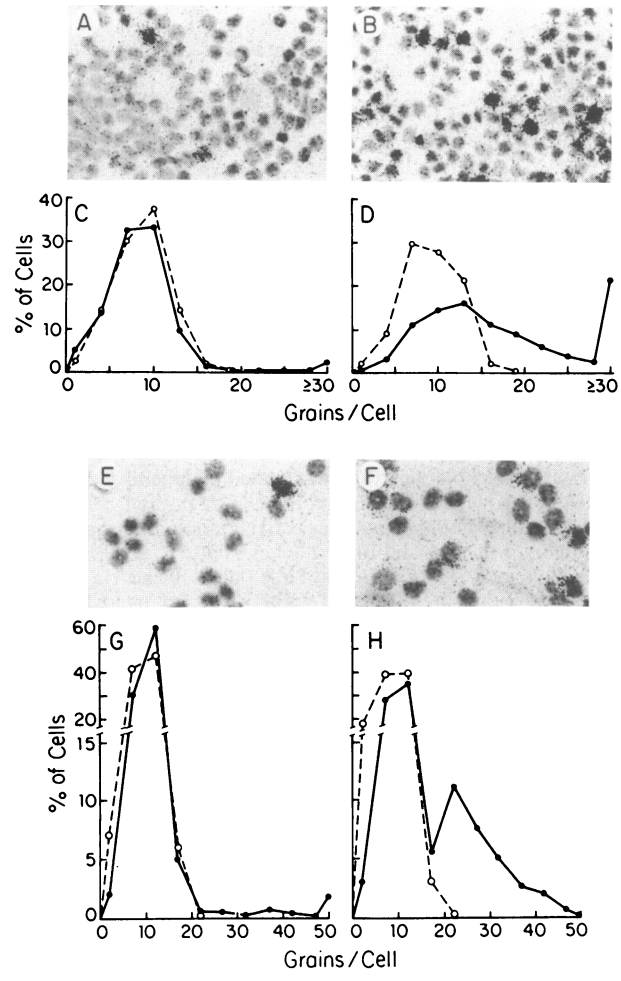

FIG. 2. Unfractionated or $CD4^+$ PB T cells hybridized *in situ* for IL-4 and IFN-γ mRNA after 6 hr of activation with ionomycin and PMA. Photomicrographs of unfractionated T cells (*A* and *B*) or $CD4^+$ T cells (*E* and *F*) hybridized with an antisense probe for IL-4 (*A* and *E*) or IFN-γ (*B* and *F*) mRNA are shown. Directly below each photomicrograph (×24) is the corresponding histogram (*C*, *D*, *G*, and *H*) of the number of grains per cell after hybridization with an antisense probe (●) for the slide depicted or with a control sense probe (○) by use of a duplicate slide processed in parallel. The slides hybridized for IL-4 mRNA were exposed for either 9 days (*A*) or 35 days (*E*), whereas the slides hybridized for IFN-γ mRNA were exposed for either 9 days (*B*) or 7 days (*F*).

markedly enhance $CD8^+$-mediated cellular cytotoxicity (4). The $CD8^+$ murine T-cell-restricted production of IL-4 we found in unfractionated circulating T cells and T-cell subsets was also true for tonsillar T cells, suggesting that this restriction was a general feature of IL-4 expression by peripheral T cells, including those found in lymphoid tissues.

IL-4 production by human peripheral T cells did not require prior *in vitro* priming—i.e., repeated *in vitro* treatment of T cells with activating stimuli such as antigens or mitogens. These results differ from those of several previous studies using murine lymph node or splenic T cells, in which priming was necessary before secreted IL-4 became detectable (36, 37). These differences may reflect significant differences between murine and human T cells in the regulation of IL-4 production, as suggested by the differences noted with T-cell clones, or the greater sensitivity of RNA blot and *in situ* hybridization for detection of IL-4 mRNA compared with assays for secreted IL-4 protein. The recent finding of IL-4 mRNA expression by Con A-treated unprimed murine splenocytes (38) at a frequency similar to that we observed in human T cells is most consistent with a methodologic rather

than a biologic difference. In addition, we found that concentration of culture supernatants was required for the detection of IL-4 protein secreted by activated T cells, whereas in previous studies culture supernatants were directly assayed. Nevertheless, the enhancement of IL-4 production seen after repeated priming *in vitro* suggests that the population of circulating T cells capable of producing IL-4 after primary *in vitro* activation may have previously been activated *in vivo* and represent memory T cells (39). Several lines of evidence provide indirect support for this hypothesis. (*i*) The $CD45R^-$ $CD4^+$ subset, which accounts for the preponderance of IL-4 production by circulating T cells, is enriched in cells that proliferate in response to soluble recall antigens after previous *in vivo* immunization (39). (*ii*) $CD45R^+$ T cells are converted in a unidirectional manner to a $CD45R^-$ surface phenotype after *in vitro* activation (30). (*iii*) Finally, we recently found that IL-4 production by human neonatal T cells and $CD4^+$ $CD8^-$ human thymocyte cell populations, which are presumably antigenically naive, is undetectable under conditions in which production by adult T cells is readily demonstrated (D.B.L. and C.B.W., unpublished work). Further studies will be required to determine whether previous activation is obligatory for IL-4 production by T cells.

The extent to which IL-4 and IFN-γ or IL-2 expression are mutually exclusive in primary T-cell populations is unknown. A division of $CD4^+$ T cells into mutually exclusive groups of either IL-4- or IFN-γ/IL-2-producing cells has been proposed based on patterns of lymphokine expression in cloned murine T cells (11, 12), and it has been suggested that this limitation of lymphokine expression may be important in permitting distinct lymphokine-mediated T-cell effector function (40). Although our data do not directly support or contradict this hypothesis, most human T-cell clones appear to be capable of producing both IL-4 and IFN-γ (9, 13), including the T-cell clone from which the human IL-4 cDNA was first cloned (41). Additional studies of IL-4, IFN-γ, and IL-2 production by activated $CD4^+$ and $CD4^+$ $CD45R^-$ T cells *in situ* may be instructive for testing this hypothesis of segregated lymphokine production in primary T cells.

While the lineage relationships of the cells producing IL-4, IFN-γ, and IL-2 remain to be determined, restricting IL-4 production to discrete populations of phenotypically distinct T cells may be important in limiting the effects of secreted IL-4 to a comparatively small number of responder cells. This may be particularly important for IL-4 because, unlike IL-2, specific receptors for IL-4 have been detected on most hematopoietic cell types examined (42, 43). Another recently described mechanism that may focus the effects of IL-4 is the directed secretion of this lymphokine, increasing the likelihood that IL-4 will be bound by adjacent cells (44). Probably multiple mechanisms are responsible for ensuring that the effects of T-cell lymphokines are limited to relevant responder cells, so that after T-cell activation by antigen the amplification of immune response is appropriately focused. Further studies will be needed to determine the relative importance of restricted lymphokine production in regulating the immune response.

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