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Lymphoproliferative Disease in Human Peripheral Blood Mononuclear Cell-Injected SCID Mice. IV. Differential Activation of Human Th1 and Th2 Lymphocytes and Influence of the Atopic Status on Lymphoma Development

Vincenzo Coppola,* Arianna Veronesi,* Stefano Indraccolo,** Francesca Calderazzo,* Marta Mion,* Sonia Minuzzo,* Giovanni Esposito,* Daniele Mauro,* Barbara Silvestri,* Paolo Gallo,† Paolo Falagiani,§ Alberto Amadori,2* and Luigi Chieco-Bianchi*

Intraperitoneal transfer of PBMC from EBV+ donors into SCID mice leads to high human Ig levels in mouse serum and B cell lymphoproliferative disease. As these events depend on the activation of co-injected human T cells, we addressed the behavior of the Th1 and Th2 subsets in this model. Production of IFN-γ, but not of Th2 cytokines such as IL-4, was detected in culture supernatants of PBMC stimulated in vitro with mouse splenocytes. Moreover, anti-CD3 stimulation of the human cells recovered from mice brought about IFN-γ, but not IL-4, synthesis; on the other hand, PCR and in situ hybridization analysis of ex vivo-recovered cells disclosed the presence of mRNA for both cytokines following in vitro restimulation, thus suggesting posttranscriptional regulation of IL-4 gene expression. When SCID mice were inoculated with PBMC from atopic donors, whose Th1/Th2 profile displays an imbalance toward Th2 cells, tumor development rates were lower, and tumor latency was higher, compared with those in mice injected with PBMC from normal donors. Isotypic analysis of human Ig in mouse serum showed the exclusive presence of IFN-γ-driven IgG subclasses; in addition, human IgE were low or undetectable in most cases. These findings indicate that following transfer into SCID mice, human Th1 lymphocytes undergo preferential activation, whereas Th2 function is down-regulated. Th1 lymphocytes probably are a major component in promoting EBV+ B cell expansion and tumor development; the individual Th1/Th2 profile could in part account for the as yet unexplained donor variability in tumor generation in this experimental model. The Journal of Immunology, 1998, 160: 2514–2522.

The SCID mouse injected i.p. with human PBMC has been proposed as a useful tool to explore lymphocyte function in a more physiologic setting than in vitro experiments (1, 2). Moreover, when PBMC from EBV-seropositive donors are injected, most animals develop EBV+B cell tumors of human origin (3–5), closely recalling the immunoblastic opportunistic lymphomas frequently observed in immunocompromised patients (6, 7). This model, therefore, may also constitute an advantageous means for studying the mechanisms that underlie the lymphomagenesis process in humans; lymphoma development, however, results from a complicated interplay of several factors of both donor and recipient origin (8) and is greatly favored by the presence of functional Th cells in the injected cell populations (9).

1 This work was supported in part by grants from the National Research Council (Target Projects ACR0 and FATMA), the Italian Association for Research on Cancer, MURST 60%, Istituto Superiore di Sanità (AIDS Project), and a fellowship from the Italian Association for Research on Cancer (to M.M.).
2 Address correspondence and reprint requests to Dr. Alberto Amadori, Department of Oncology and Surgical Sciences, University of Padova, Via Gattamelata 64, 35128 Padova, Italy. E-mail address: albido@ux1.unipd.it

The immunologic events that occur following SCID mouse injection with human mature lymphoid cells are complex and relatively unclear. It is known that the in vivo passage of human T cells into the xenogeneic microenvironment profoundly modifies their behavior; indeed, following initial activation against murine Ags, the transferred T cells become progressively anergic (10), as shown by their inability to proliferate and release IL-2 in response to TCR stimulation (11–13). In addition, the progressive shaping of the T cell repertoire toward xenoreactivity (11) is accompanied by a prominent B cell activation; in this case as well, the human B cell response seems to be monopolized by anti-mouse Ag specificities (14, 15).

Th lymphocytes are phenotypically and functionally heterogeneous, and their products are numerous and often pleiotropic. In both mice (16) and humans (17), a functional dichotomy into Th1 and Th2 cells has been advanced; Th1 lymphocytes produce cytokines that mainly favor cellular immunity, such as IL-2 and IFN-γ, whereas Th2 cells synthesize cytokines (IL-4, IL-5, and IL-6) that mainly co-operate with B lymphocytes in humoral responses. However, the original dichotomy made in the mouse (16) is probably less stringent in humans, where a continuous distribution of T cell populations endowed with an almost infinite spectrum of cytokine combinations is probably closer to reality, and exclusive IFN-α or IL-4 production identifies T cell clones strongly polarized toward Th1 or Th2 phenotypes, respectively (18). In any case, surface markers to identify these different populations are not available, and CD30 Ag expression does not represent an unequivocally distinctive feature of human Th2 lymphocytes (19, 20). The aim of this study was to assess the behavior of...
human Th1 and Th2 lymphocytes in the SCID mouse experimental model by assessing the relative activation of IFN-γ and IL-4-producing cells following stimulation by murine heteroantigens.

Materials and Methods

Study population

Informed consent was obtained from 26 adult volunteers undergoing lymphapheresis; prior EBV infection was confirmed by serologic tests for antiviral capsid Ag and anti-nuclear Ag IgG. Nineteen healthy blood donors had no history of allergic diseases; seven subjects, of comparable age and sex distribution, were atopic, as evidenced by anamnestic, clinical, and laboratory findings. Three of these individuals had serum IgE specific for polli, two had serum IgE specific for both pollen and house dust mites, and one had serum IgE specific for mites only; one subject had severe atopic eczema and multiple reactivity against several allergens. This last case had serum IgE levels exceeding 11,500 IU/ml, while levels in most of the other atopic individuals (mean, 208 ± 149 IU/ml; range, 100–460) were within the normal range (mean, 103 ± 32; range, 60–148).

Cell preparation

PBMC were isolated by Ficoll-Hypaque (Pharmacia-LKB, Uppsala, Sweden) gradient centrifugation as reported previously (21, 22). The cells were washed three times; counted; resuspended in RPMI 1640 medium supple-
mented with 10% FCS (Life Technologies, Grand Island, NY), 1% nonessential amino acids, 1% l-glutamine, and 2 x 10^{-5} M 2-ME (complete RPMI); and either injected as such into SCID mice or employed in in vitro experiments.

Mouse injection

Nonleaky SCID mice were purchased from IFFA Credo (L’Aberiselle, France) and maintained in our animal facilities under pathogen-free conditions (9). Groups of 7–9 wk-old mice of both sexes were inoculated i.p. with 80 to 100 x 10^6 unfractinated PBMC. Before injection and every 10 days thereafter, the mice were bled from the retro-orbital plexus to

In vitro cytokine production and assay

To evaluate cytokine production, freshly isolated PBMC were cultured in the presence of murine spleenocytes or anti-CD3 mAb. Briefly, the cells (1 x 10^9 cells/ml in RPMI medium) were incubated in the wells of U-bottom microtiter plates (Costar, Cambridge, MA) with equal numbers of 3000 rad-irradiated spleen cells from BALB/c mice (which have the same genetic backbone as SCID animals) or with anti-CD3 mAb (provided by F. Malavasi, Turin, Italy; 100 ng/ml final concentration). The cultures were harvested at different time intervals; cell-free supernatants (SN) were re-
covered by low speed centrifugation and stored at -20°C until testing.

To assess the ability of the human cells recovered from PBMC-injected animals to produce different cytokines, the cells were resuspended in complete RPMI (1 x 10^7/ml) and cultured in the absence and the presence of anti-CD3 mAb; in view of the virtual absence of accessory cells in ex vivo-recovered populations, the cells were co-cultured with anti-CD3 mAb previously coupled to the wells of polystyrene microtiter plates (no. 3072, Falcon, Grenoble, France; 10 μg/ml in carbonate-bicarbonate buffer, pH 9.6). After different time periods, cell-free SN were collected and assayed for IFN-γ and IL-4 contents by ELISA (BioSource International, Cama-
rillo, CA; and Medegenics Diagnostics, Fleurus, Belgium, respectively). Re-
tults were expressed as picograms per milliliter; the sensitivity limits of the assays for IFN-γ and IL-4 detection were both 1 pg/ml.

Cytolourographic analysis of cytokine production

Two-color cytolourographic analysis of intracellular human IFN-γ and IL-4 synthesis in freshly isolated PBMC and ex vivo-recovered cells was conducted as previously described (23). Briefly, the cells (1 x 10^6) were stimulated with solid phase anti-CD3 mAb in the presence of 1 μM mono-

PCR for cytokine mRNA analysis

Total RNA was extracted using the RNAzol B method (Biotex Laborato-
ries, Houston, TX) as previously described (24). One microgram of total RNA was used for the synthesis of first-strand cDNA using reverse tran-
scriptase (Life Technologies, Gaithersburg, MD) and oligodT(18) primer, ac-
nording to the manufacturer’s instructions. The following primers were used in PCR to amplify human IL-4, IFN-γ, and CD3γδ transcripts: IL-4 forward, 5'-CAT TGG GCT CTT CGT TCT CTC CAT A-3'; IL-4 reverse, 5'-CGA ACA CTA TTA TTT CTC TCT CAT C-3'; IFN-γ forward, 5'-GCA TGG TTT GGT GTC TTC GCT ACC GC-3'; IFN-γ reverse, 5'-CTC CTT CTT CGC CTT CTT TGA GAC GG-3'; CD3γδ forward, 5'-CGG AGA GAC GAG GCT AGA TGA GAA C3-3'; CD3γδ reverse, 5'-GTC ATC TCC TGG ACC GAC CCT TCT CAT A-3'. IL-4 primers amplify a product of 456 bp from cDNA (GenBank accession no. M13982); IFN-γ primers amplify a 427-bp product from cDNA (GenBank accession no. X13274); finally, CD3γδ primers amplify a 0.8-kb product from cDNA (GenBank accession no. X60626, CD3γδ forward; GenBank accession no. X60632, CD3γδ reverse). In no case was a PCR product obtained from human genomic DNA under our conditions.

To quantify the amplified products, coamplification was performed with 5'-3'-P-labeled reverse primers. The amplified products were separated on 5% acrylamide gels, dried, exposed to x-ray film, and analyzed by an Ultrascan XL enhanced laser densitometer (Pharmacia-LKB); the data were per-
centially expressed as the amount of the IL-4 or IFN-γ transcript over the amount of the CD3γδ transcript amplified from the same cDNA sample.

In situ hybridization (ISH)

ISH was performed as reported previously (25). Briefly, ex vivo-recovered cells, either immediately or following overnight solid phase anti-CD3 activation, were resuspended to 1 x 10^6 cells/ml in RPMI medium and centrifuged on slides using a Cytospin (Shandon, Runcorn, U.K.) centri-
fuge. Slides were air-dried, fixed in acetone, and stored at -80°C until use. Before processing, the slides were postfixed in PBS–4% paraformaldehyde, rinsed in PBS, and treated with 0.1 M triethanolamine, pH 8.0, and 0.25% acetic anhydride before ethanol dehydration. Hybridization was performed for 16 h at 50°C. The hybridization mixture (20 μl over each cellular spot) contained 2 x 10^6 cpm [35S]UTP-radiolabeled antisense RNA probe specific for mRNAs coding for human IFN-γ or IL-4 as described previously (25); for the control, the slides were also hybridized with the corresponding sense probe. After washing, the slides were treated with RNase A (Boehr-
inger Mannheim, Mannheim, Germany; 10 mg/ml), rinsed, dehydrated again in 95% ethanol, developed, and finally air-dried. For autoradi-
ographic detection, the slides were dipped into NBT-2 emulsion (Eastman

Abbreviations used in this paper: SN, supernatants; ISH, in situ hybridization.
Kodak, Rochester, NY), exposed in dark with desiccant at 4°C for various time intervals, and finally counterstained with Mayer hematoxylin. IFN-γ and IL-4 mRNA-expressing cells were counted blindly and independently by two investigators on three different slides from the same sample; cells containing >20 grains (or in any case >4 times the background level in the sense sample) were scored as positive, and the results were expressed as a percentage of positive cells. In each experiment, the activity and specificity of both probes were also tested, as positive and negative controls, on two cell lines: a human IL-4-transfected melanoma cell line (provided by Dr. M. P. Colombo, Milan, Italy), and a human IFN-γ-transfected mouse cell line (courtesy of Dr. H. Young, Frederick, MD).

Human Ig assays

Human IgG levels in donor and PBMC-inoculated mouse sera were evaluated by RIA as detailed previously (26). Human total and specific IgE levels were determined by solid phase RIA (Serikit total IgE and Serikit specific IgE; respectively; Lofarma, Milan, Italy) according to the manufacturer’s instructions. The isotype of the human oligoclonal IgG bands present in mouse serum was studied by isoelectrofocusing and Western blotting as reported previously (27) with minor modifications; antisera to specific IgE, respectively; Lofarma, Milan, Italy) according to the manufacturer’s instructions.

Statistical analysis

Unless otherwise specified, results were expressed as the mean ± SD; data were analyzed using the Wilcoxon paired test, Mann-Whitney U test, and two-tailed Fisher’s exact test, where appropriate.

Results

Cytokine production by mouse Ag-activated PBMC

We first studied the profile of the cytokines produced in vitro by human T lymphocytes in response to murine xenogenants; to this end, we chose IFN-γ and IL-4, as their production characterizes cells strongly polarized toward Th1- and Th2-like functions, respectively (18). As a control, PBMC were also stimulated with a polyclonal T cell activator such as anti-CD3 mAb. Following 48-h in vitro stimulation with mouse spleen cells (Table I), freshly isolated PBMC from healthy donors produced measurable amounts of IFN-γ, which were significantly higher upon activation with anti-CD3 (p < 0.05, by Wilconox’s test). On the contrary, IL-4 synthesis was not detectable following xenogenantigenic stimulation in any of the individuals tested (Table I) even when culture SN were assayed for IL-4 contents at different culture times (from 24 to 144 h; data not shown). It is known that in vitro activation of freshly isolated PBMC is associated with a poor IL-4 response (20); accordingly, low levels of IL-4 could be evidenced in culture SN in most cases following polyclonal PBMC activation (Table I). When we tested the response of PBMC from atopic patients, who show an imbalance toward the Th2 subset in their peripheral Th1/Th2 profile (28, 29), both mouse Ags and anti-CD3 mAb elicited a measurable IFN-γ response, which did not differ significantly from that of healthy controls (Table I). Cocultivation of PBMC from atopic patients with murine splenocytes was not associated with IL-4 synthesis in any of the individuals tested; as expected, IL-4 production in response to polyclonal T cell activation was significantly higher than that in healthy controls (Table I; p < 0.05, by Mann-Whitney U test).

Cytokine production by in vivo-passaged cells

We next addressed the ability of cells from healthy donors to produce IFN-γ and IL-4 following in vivo passage into SCID mice. After ex vivo recovery, in most experiments no spontaneous production of either cytokine could be detected in the SN of freshly recovered, unstimulated cells (Table II); in some instances, instead, a sizable IFN-γ production could be detected (Expt. 6 in Table II). Following in vitro, solid phase, anti-CD3 activation, the recovered cells did not produce IL-4, whereas they were able to release substantial IFN-γ amounts (Table II); nonetheless, PMA/ionomycin treatment of the cells resulted in substantial production of both cytokines (not shown). When ex vivo-recovered cells were cultured in the presence of rIL-2 for 30 days, they maintained their ability to produce IFN-γ and gained the ability to produce IL-4 following in vitro anti-CD3 stimulation (Table III). These findings partly confirmed previous observations (13) and seemed to indicate that a reversible anergic status mainly concerned Th2-like functions, such as IL-4 production.

### Table I. Cytokine production by human PBMC following in vitro stimulation with murine splenocytes or anti-CD3 mAb

<table>
<thead>
<tr>
<th>Donor No.</th>
<th>Mouse cells</th>
<th>Anti-CD3 Mouse cells</th>
<th>Anti-CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>140</td>
<td>&gt;1200</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2</td>
<td>156</td>
<td>1000</td>
<td>&lt;1</td>
</tr>
<tr>
<td>3</td>
<td>536</td>
<td>540</td>
<td>&lt;1</td>
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<td>4</td>
<td>96</td>
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<tr>
<td>5</td>
<td>40</td>
<td>&gt;1200</td>
<td>&lt;1</td>
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<tr>
<td>6</td>
<td>28</td>
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</tr>
<tr>
<td>7</td>
<td>56</td>
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</tr>
<tr>
<td>8</td>
<td>64</td>
<td>392</td>
<td>&lt;1</td>
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<tr>
<td>Atopic</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>&gt;1200</td>
<td>&lt;1</td>
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<tr>
<td>15</td>
<td>72</td>
<td>192</td>
<td>&lt;1</td>
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</tbody>
</table>

### Table II. In vitro cytokine production by in vivo passaged human lymphoid cells

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Unstimulated</th>
<th>Anti-CD3</th>
<th>Unstimulated</th>
<th>Anti-CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;1</td>
<td>67</td>
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<td>2</td>
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<td>4</td>
<td>&lt;1</td>
<td>115</td>
<td>&lt;1</td>
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</tr>
<tr>
<td>5</td>
<td>&lt;1</td>
<td>54</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>6</td>
<td>142</td>
<td>523</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

### Table III. In vitro cytokine production by human lymphoid cells recovered from SCID mice before and after culture with rIL-2

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Cells</th>
<th>IFN-γ (pg/ml)</th>
<th>IL-4 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Freshly recovered</td>
<td>&lt;1</td>
<td>205</td>
</tr>
<tr>
<td>rIL-2 cultured</td>
<td>3</td>
<td>52</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2</td>
<td>Freshly recovered</td>
<td>&lt;1</td>
<td>123</td>
</tr>
<tr>
<td>rIL-2 cultured</td>
<td>2</td>
<td>91</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*a Unfractionated PBMC from healthy and atopic donors were cultured in vitro in the presence of BALB/c splenocytes or anti-CD3 mAb as detailed in Materials and Methods. Cell-free SN were recovered after 48 h and tested for IFN-γ and IL-4 content.

*b Human lymphoid cells from healthy donors were recovered by peritoneal washing 4 wk after transfer into SCID mice and cultured in the absence or presence of solid-phase anti-CD3 mAb as detailed in Materials and Methods. Cell-free SN were recovered 48 h later and tested for IL-4 and IFN-γ content.
Cytofluorographic analysis of cytokine production by in vivo-passaged cells

To exclude the possibility of underestimating cytokine contents in culture SN due to cytokine consumption by activated cells bearing the appropriate receptor and/or the low number of human CD3+ lymphocytes in ex vivo-recovered cells, we also evaluated the percentage of cells showing intracellular production of a given cytokine by cytofluorographic analysis. As shown in Figure 1, a small fraction of freshly isolated human CD3+ cells produced either IFN-γ or IL-4 in response to in vitro anti-CD3 stimulation; these figures agree with previous data (23, 30). Four weeks after transfer into SCID mice, the recovered cells did not spontaneously produce human IFN-γ or IL-4 in the absence of mitogenic stimulation (Fig. 1); following overnight stimulation with solid phase anti-CD3 mAb, a small fraction of ex vivo-derived CD3+ lymphocytes was positive to the anti-IFN-γ mAb, whereas the number of IL-4-producing cells was negligible (Fig. 1). The results of an experiment comparing cytofluorographic analysis and culture SN evaluation are summarized in Table IV.

Cytokine RNA message in in vivo-passaged cells

The differential ability of ex vivo-recovered cells to produce IFN-γ and IL-4 prompted us to study the cytokine RNA message in these cells by PCR and ISH techniques. A typical experiment comparing cytokine production in culture SN and mRNA expression by PCR is shown in Figure 2. The IFN-γ and IL-4 transcripts were barely detectable in freshly isolated PBMC (not shown), and in vitro stimulation with anti-CD3 was associated with strong expression of both messages (Fig. 2, top, lanes b and f) as well as with detectable cytokine production in culture SN (Fig. 2, bottom). On the other hand, following in vivo passage, in most experiments freshly recovered cells did not show a significant transcription of the IFN-γ and IL-4 genes, which were both strongly reactivated following in vitro, solid phase, anti-CD3 stimulation (Fig. 2, top, lanes d and h);

![Diagram](https://example.com/diagram.png)

**FIGURE 1.** Two-color cytofluorographic analysis of cytokine production in PBMC before and after in vivo passage into SCID mice. Freshly isolated PBMC from healthy donors were tested for their ability to produce IFN-γ and IL-4 following anti-CD3 stimulation before (top panels) injection into SCID animals. Four weeks after transfer, the cells were recovered by peritoneal washing and tested either immediately for intracytoplasmic cytokine contents (bottom left panels) or following 48-h anti-CD3 stimulation (bottom right panels). The human CD3+ cells were gated by surface staining with anti-CD3 mAb, as detailed in Materials and Methods. One representative experiment is shown; the percentage of CD3+ IFN-γ+ or CD3+ IL-4+ lymphocytes is indicated in each panel.

<table>
<thead>
<tr>
<th></th>
<th>Before Injection</th>
<th>Ex Vivo Recovered</th>
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<tbody>
<tr>
<td></td>
<td>IFN-γ</td>
<td>IL-4</td>
</tr>
<tr>
<td>% positive cells</td>
<td>Unstimulated</td>
<td>Anti-CD3</td>
</tr>
<tr>
<td>&lt;1</td>
<td>9.6</td>
<td>&lt;1</td>
</tr>
<tr>
<td>pg/ml</td>
<td>3</td>
<td>352</td>
</tr>
</tbody>
</table>

* PBMC were tested for the ability to produce cytokines before transfer into SCID mice and following ex vivo recovery; IFN-γ or IL-4 synthesis was evaluated both by cytofluorographic analysis and in culture SN. Results from a representative experiment are shown.
as outlined above, however, culture SN analysis could only demonstrate the translation of the IFN-γ mRNA, whereas the IL-4 message was not apparently associated with cytokine production (Fig. 2, bottom).

The PCR data obtained in five consecutive experiments with PBMC from healthy donors are summarized in Table V, where, to overcome the different percentages of CD3⁺ cells in the different experimental samples, data were evaluated by densitometric analysis, and the results were expressed as a percentage of the CD3 band. In freshly isolated PBMC, both IFN-γ and IL-4 mRNA were clearly demonstrable in all the experiments following anti-CD3 stimulation. In ex vivo-recovered cells, the cytokine message was usually negligible immediately after recovery, even though a significant mRNA expression for IFN-γ (Expt. 1 in Table V) or IL-4 (Expt. 2) was occasionally detected in unstimulated cells. Following anti-CD3 stimulation, the IFN-γ message was invariably expressed, as was the IL-4 transcript in several cases (Expts. 2 and 3 in Table V).

![Figure 2](http://www.jimmunol.org/bym/)

**FIGURE 2.** Comparative analysis of cytokine mRNA expression and cytokine release in culture SN of freshly isolated PBMC and of cells recovered after in vivo passage into SCID mice. The upper panel represents PCR results of IFN-γ (lanes a–d, bottom) and IL-4 (lanes e–h, bottom) mRNA in the different experimental samples compared with CD3γ mRNA expression in the same samples (lanes a–h, top). The bars in the lower part of the figure represent the amounts of cytokine produced in culture by the cells in the different experimental conditions. Results from a representative experiment with PBMC from a healthy donor are presented.

These findings seemed to envisage the existence of a post-transcriptional control of IL-4 secretion. To confirm these data, we also studied cytokine mRNA expression in ex vivo-recovered cells by ISH analysis; the specificity of the probes used is illustrated in Figure 3 (A–D). In most experiments, the percentage of ex vivo-derived cells bearing IFN-γ and IL-4 messages was negligible (Fig. 3, E and F, respectively); following in vitro activation, transcription of the IFN-γ, but not the IL-4, gene could be detected in about 30 to 50% of the cells (Fig. 3F). In a few cases, a strong positivity for IL-4 mRNA was also evident in most cells immediately after their recovery from the SCID mouse (Fig. 3G), and this message was only slightly changed by in vitro anti-CD3 mAb stimulation (Fig. 3H). Together, these observations seemed to strongly indicate that the IL-4 secretion could be blocked at a post-transcriptional level in ex vivo-recovered cells.

**Tumor development in SCID mice injected with PBMC from atopic and nonatopic donors**

As mentioned above, the generation of lymphomatous lesions in PBMC-injected SCID mice greatly depends on T cell functional help (8, 9). In view of the above findings, we wondered whether a different Th1/Th2 profile within the peripheral lymphoid compartment could influence tumor development in this experimental model. To date, it is unexplained why tumor prevalence and latency vary greatly when PBMC from different donors are injected (31). Since allergic patients show an imbalance toward Th2 cells in their peripheral Th1/Th2 profile (28, 29), we addressed the effect of donor allergic status on tumor development. As shown in Table VI, following i.p. injection of unfractionated PBMC from 9 normal subjects, 48 of 57 mice developed tumors (84%), while PBMC from 7 allergic patients led to tumor development in 19 of 34 injected animals (55.9%; p = 0.003). Moreover, when atopic patients were arbitrarily categorized as low or high IgE donors according to their serum IgE contents (<300 and >300 IU/ml, respectively), lymphoma development was observed in only 2 of 13 SCID mice injected with PBMC from high IgE patients (15.4%) and in 17 of 21 mice injected with PBMC from low IgE patients (80.9%; p < 0.001); this latter incidence did not differ significantly from the figures obtained with PBMC from nonatopic subjects (Table VI). Tumor latency as well differed in tumor-bearing mice injected with PBMC from allergic and nonallergic donors (Table VI); lymphomas developed more rapidly in mice inoculated with PBMC from normal individuals (mean latency, 7.7 ± 2.1 wk; range, 4–12) than in mice receiving PBMC from atopic patients (10.6 ± 4.6 wk; range, 6–22; p = 0.012).

**Analysis of human IgG and IgE isotypes in serum of PBMC-injected SCID mice**

To better substantiate the above data, we explored the behavior of human Ig in the serum of SCID animals injected with PBMC from atopic and nonatopic donors. The kinetics of human IgG release in mouse serum differed in the two donor groups, and as early as 10 days after cell transfer, detectable IgG levels were found only in mice injected with PBMC from healthy individuals (not shown). Forty days after cell transfer, the IgG values found in 23 animals injected with PBMC from allergic patients were significantly lower than those observed in 21 animals receiving PBMC from nonallergic subjects (mean ± SEM, 6.6 ± 1.5 vs 14.7 ± 3.1, respectively; p = 0.006). Furthermore, since PBMC transfer is associated with the appearance of a distinct oligoclonal human IgG pattern in the serum of the injected animals (32, 33), we studied the isotypic profile of these oligoclonal bands. As shown in Figure 4, mainly human IgG1 bands were evident in mouse serum 40 days after PBMC transfer from all the donors, and the intensity of the bands was higher in the sera from nonatopic donors (Fig. 4A) than in sera from atopic donors (Fig. 4B). No IgE bands were detectable in any of the sera tested.

**Table V.** PCR analysis of cytokine mRNA expression in freshly isolated PBMC and in human cells recovered from SCID mice

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Freshly Isolated PBMC</th>
<th>Ex Vivo Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IFN-γ</td>
<td>IL-4</td>
</tr>
<tr>
<td>1</td>
<td>2³</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>5.3</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>2.8</td>
<td>15</td>
</tr>
</tbody>
</table>

³ PBMC from healthy donors were analysed for cytokine mRNA expression before injection into SCID mice and following ex vivo recovery.

Results were expressed as percentage of value compared with the densitometric value, corresponding to the CD3γ mRNA band (=100%), as detailed in Materials and Methods.
after PBMC transfer, with only minor traces of IgG3. These findings also were compatible with the above results, because it is known that the isotypic shift toward IgG1 and IgG3 in humans depends on IFN-γ, whereas the production of different IgG subclasses is promoted by IL-4 and other Th2-derived cytokines.

Finally, as far as the human IgE isotype was concerned, 40 days after i.p. inoculation of unfractionated PBMC from normal donors, no human IgE could be detected in the serum of 50 animals (not shown). On the other hand, when PBMC from atopic subjects were injected, detectable human IgE levels could be evidenced 40 days later in the serum of only 50% of mice (Fig. 5); their levels, however, in most cases were 1 to 2 logs lower than donor serum values, whereas IgG values in most cases approached donor serum levels (Fig. 5). Kinetic studies in animals injected with PBMC from a patient with very high serum IgE (11,500 IU/ml) showed that the gap between human IgG and IgE levels in mouse serum remained constant throughout the entire follow-up period; detectable IgE levels could be demonstrated only very late after cell transfer (Fig. 6) and at much lower levels compared with donor serum values. These findings, which were similar to data incidentally recorded by

![FIGURE 3.](image1.png)

**FIGURE 3.** In situ hybridization analysis of IFN-γ and IL-4 mRNA expression in ex vivo-recovered human cells. The cells recovered by peritoneal washing 4 wk postinjection into SCID mice of PBMC from a healthy donor were analyzed by ISH with IFN-γ and IL-4-specific probes. A and B show the pattern of a human IFN-γ-transfected mouse cell line (sense and antisense probes, respectively); C and D depict the pattern of a human IL-4-transfected melanoma cell line (sense and antisense probes). E and F represent unstimulated and anti-CD3-stimulated cells after ex vivo recovery, respectively (antisense IFN-γ probe), whereas G and H refer to the same samples hybridized with the IL-4-specific antisense probe. The percentages of positive cells in each experimental condition were 2% (E), 45% (F), 53% (G), and 58% (H).

![FIGURE 4.](image2.png)

**FIGURE 4.** Isotypic profile of human oligoclonal IgG bands in the serum of SCID mice injected with PBMC. Forty days following transfer of 100 × 10⁶ unfractionated PBMC from a normal donor, the isotype of the human oligoclonal IgG bands present in mouse serum was investigated by isoelectrofocusing, as detailed in Materials and Methods. The arrow indicates the site of sample application on agarose gel.

<table>
<thead>
<tr>
<th>Patient Code</th>
<th>Serum IgE (IU/ml)</th>
<th>Tumor-Bearing/Injected Mice</th>
<th>Latency (no. wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>120</td>
<td>4/8</td>
<td>8; 10; 10; 22</td>
</tr>
<tr>
<td>ZM</td>
<td>325</td>
<td>1/3</td>
<td>19</td>
</tr>
<tr>
<td>DBM</td>
<td>105</td>
<td>3/3</td>
<td>9; 10; 11</td>
</tr>
<tr>
<td>LL</td>
<td>100</td>
<td>4/4</td>
<td>11; 12; 14</td>
</tr>
<tr>
<td>BE</td>
<td>11,500</td>
<td>1/4</td>
<td>17</td>
</tr>
<tr>
<td>FF</td>
<td>460</td>
<td>0/6</td>
<td>—</td>
</tr>
<tr>
<td>GF</td>
<td>140</td>
<td>6/6</td>
<td>6; 6; 6; 6; 7</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>103 ± 32^a</td>
<td>48/57</td>
<td>7.7 ± 2.1 (4–12)^c</td>
</tr>
</tbody>
</table>

^a Groups of SCID mice were injected i.p. with 80–100 × 10⁶ unfractionated PBMC from seven atopic or nine nonatopic donors and followed as detailed in Materials and Methods.

^b Mean value ± SD.

^c Mean value ± SD (range in parentheses).
other workers (14) with no further explanation, also were compatible with the hypothesis of a preferential activation of human Th1 lymphocytes within the SCID mouse microenvironment.

Discussion

These data confirm and extend previous work (9, 11–13) and indicate that human T cell activation in the SCID mouse model is a nonrandom phenomenon differentially involving T cell functional subsets, as demonstrated by 1) the ability of in vitro mouse Ag-stimulated PBMC and in vivo-passaged human cells to produce IFN-γ but not IL-4; 2) the predominance of IFN-γ-driven human oligoclonal IgG in the serum of PBMC-injected SCID mice, and 3) the differential behavior of human IgE and IgG in mouse and donor sera. Our data suggest that Th1 lymphocytes undergo preferential activation within the xenogeneic microenvironment, whereas Th2-like functions are selectively modulated. The finding that human T cell activation in the SCID mouse model selectively involves different functional T cell subsets has several implications and deserves much attention.

It is conceivable that the in vitro inability of PBMC to synthesize IL-4 in response to murine Ags could be due to a lack of cellular interactions required for Th2 cytokine production. It is known that IL-4 production not only depends on TCR engagement by the relevant Ag, but also on a number of additional interactions provided by APC. Indeed, several different factors may influence the orientation of T cells toward either the Th1 or the Th2 pathway (34), including the surrounding cytokine microenvironment (35), the affinity of TCR/MHC interaction (36), and the activation of different costimulatory pathways (37–40). On the other hand, a body of evidence indicates that the Th1 phenotype could be a default activation pathway in the absence of specific signals driving differentiation toward the Th2 phenotype (41–43). Our data do not help discern whether murine xenogenigs preferentially promote pathways leading to IFN-γ rather than IL-4 production or simply do not provide the necessary cosignals to drive Th2 differentiation.

In reference to the modulation of Th2-like functions observed in ex vivo-recovered human cells, our findings extend and partially confirm data reported by Saxon and co-workers (13), who demonstrated that in vivo-passaged cells did not proliferate or produce IL-2 in response to TCR stimulation; we instead observed a maintained capacity to produce IFN-γ following anti-CD3 stimulation. This discrepancy may be explained by several factors, such as the different experimental conditions employed and the different cytokines studied. We examined the behavior of IFN-γ, the production of which better characterizes polarization toward the Th1 phenotype; IL-2 synthesis could not completely discriminate between Th1 and Th2 lymphocytes, because it is a product of Th2 cells as well (44). In addition, we recovered cells relatively early following i.p. transfer, and T cell tolerization might not have proceeded to a fully anergic status; nonetheless, the induction of T cell anergy by supraimmunogenic doses of Ag is generally a very early phenomenon (45). In any case, our data suggest that induction of a reversible anergic status in Th2 cells could be an earlier event than the tolerization of Th1-like functions.

The pathways leading to this Th2 anergic status are unclear. Since IL-4 synthesis in ex vivo-recovered cells was explored both in culture SN and at the cellular level, it is improbable that the observed lack of IL-4 production following mouse Ag activation is artifactual. Thus, our findings of no IL-4 secretion in association with a clear activation-induced message for IL-4 would seem to indicate that post-transcriptional events modulate mRNA translation in in vivo-passaged cells. While a large body of information is available on transcriptional control of cytokine genes, in particular IL-4 (reviewed in Ref. 46), little is known regarding the post-transcriptional control of cytokine synthesis; to date, the uncoupling of cytokine mRNA expression and protein secretion has been mainly reported in humans for TNF-α and some C-C chemokines (47). A similar event could occur for other cytokines, either by a translational block that prevents protein synthesis or by increased mRNA degradation before protein synthesis (45). Our findings
buttress the idea that inhibition of cytokine mRNA expression might not be a general feature of T cell anergy induction, as shown in several experimental models (48–50), and that under particular conditions cytokine production during the induction phase of anergy may also be controlled by post-transcriptional mechanisms. In other words, inadequate T cell activation by mouse xenogenotigs could translate into abortion of Th2-like functions, where IL-4 gene activation is not followed by physiologic translation of the relevant message.

Our data help in understanding some as yet unexplained observations in the SCID mouse model. Other workers found that PBMC from individual donors differ in their abilities to cause tumors in SCID mice; this variability, however, apparently does not reflect the number of circulating EBV+ B cell precursors (31, 51). The present data suggest that the peripheral Th1/Th2 profile could constitute a possible factor underlying this phenomenon, and that IFN-γ and/or other Th1-type cytokines are a major element in promoting B cell expansion and eventual tumor generation in this experimental model. We previously demonstrated (9) that the presence of human Th lymphocytes within the cell inoculum was strictly required for EBV+ B cell precursor expansion and progression to tumor. Since an imbalance between Th1 and Th2 subsets in atopic subjects is well known (28, 29), it is conceivable that, due to the lower number and/or function of the Th1 compartment (28, 29, 52), PBMC from allergic subjects could undergo lesser activation following transfer into SCID mice compared with that from nonatopic donors; this event may translate into a lower efficiency and a greater delay in EBV+ B cell expansion and eventual tumor generation. The finding of significantly lower human IgG values in the serum of animals injected with PBMC from atopic donors vs that of animals receiving PBMC from nonallergic subjects as well as the behavior of human IgE are consistent with this idea.

In this regard, our findings might also be relevant to the lymphomagenesis process in man. Stimulating, but nonconclusive, epidemiologic data suggest a lower incidence of malignant B cell lymphoproliferative disorders among atopic subjects (53–55). Indeed, lymphoma development in the SCID mouse model relies on a complex interplay of different factors (8, 56), including, as shown here, the balance between the two major cytokine-producing T cell subsets. B cell lymphomagenesis in man as well is a very complicated process (57), where not only EBV infection and immunodeficiency, but also chronic T cell stimulation could act in a coordinated manner to cause lymphoma establishment (58); it is noteworthy that some low grade B cell lymphomas arising in gut-associated lymphoid tissues are strongly dependent on continuous stimulation by products of intramucosal T cells activated by Helicobacter pylori Ags (59). Further investigations would help to understand whether our present experimental observations could be related in some manner to the population findings on lymphoma prevalence among atopic patients (53–55).

Last, but not least, our data may caution against the use of the SCID mouse model in human lymphocyte function studies. It was known that T cells injected into SCID animals undergo strong activation, and that the original repertoire is overwhelmed by the expansion of mouse-specific T and B cells (10–15). The selective activation or down-regulation of T cell subsets endowed with special functions reported here might represent an additional, significant bias that could influence the outcome of experimental procedures, such as subsequent challenge of the transferred cells with Ags or infectious agents (60–62) and, at least in some conditions, preclude the transfer of the conclusions to a specific physiopathologic setting. This problem would seem particularly important in the case of HIV infection, in view of the pivotal role played by the cytokine network in the immunopathogenesis of AIDS (63, 64).

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