AFRICAN-EUROPEAN DIFFERENCES IN THE CAPACITY OF T-CELL CYTOKINE PRODUCTION

ASTRID WILFING, STEFAN WINKLER, KARL SCHRATDBAUER, MARTIN WILLHEIM, KARIN BAIER, ALEXANDER AICHELBURG, THOMAS MÜLLER, WOLFGANG GRANINGER, AND PETER G. KREMSNER

Department of Internal Medicine I, Division of Infectious Diseases, Institute of General and Experimental Pathology, and Department of Pediatrics, University of Vienna, Vienna, Austria; Research Unit of the Albert Schweitzer Hospital, Lambarene, Gabon; Department of Parasitology, Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany

Abstract. Regional differences in immune responsiveness have been studied by comparing the frequency of cytokine producing T cells in healthy African children and adults and their age-matched European counterparts. By use of flow cytometry for the intracellular detection of cytokines an overall expansion of CD4+ and CD8+ T cells producing the Type 1 cytokines interleukin (IL)-2 and interferon (IFN)-γ was observed in adults when compared with children, giving credit to the cumulative effect of contacts with environmental antigens. The CD4+ cells expressing the Type 2 cytokines IL-4 and IL-13, however, increased only in Africans, probably reflecting continuously present challenges with antigens that preferentially drive Type 2 responses. A striking increased frequency of both Type 1 and Type 2 cytokines producing T cells was found in African adults when compared with their European counterparts. The quantitative and qualitative regional differences in immune reactivity are likely to be of significance for all immune intervention strategies, especially for the design of vaccines.

INTRODUCTION

The impact of frequently encountered contacts with infectious agents on immune responsiveness has been the subject of debate in recent years. Some immunoparasitological studies suggested a link between the decline of childhood infections and the continuously increasing frequency of hyper-sensitivity disorders and autoimmune disease in the developed world.5 These findings would require substantial and obviously acquired differences in immune responses between those incessantly exposed to a great variety of pathogens—for example, natives of tropical Africa and those only sporadically exposed to a restricted number of microorganisms, such as Central Europeans.

However, the immune responsiveness of people differentially exposed to environmental antigens has never been directly compared, although considerable consequences with respect to immune intervention strategies, including the development of vaccines, can be expected. According to the assumption that the number of infections acquired throughout the lifetime will have measurable effects on the immune system, we hypothesized that the differences in immune presentation between differentially exposed groups would be more pronounced in adults than in children because differences in the cumulative antigen-dose are likely to increase with age. This has been shown recently for African patients with malaria, who showed a striking age-dependent increase in the frequency of cytokine-producing T cells, possibly contributing to the development of antimalarial protection.6

To best investigate the impact of the number of encountered infections on the presentation of the immune system, we focused on T cells and their products, because they are essential in mediating host immune responses to a multitude of microorganisms by regulating specific antibody formation and by inducing antibody-independent immunity. T cells can be divided into at least 2 functional subsets, interferon (IFN)-γ and interleukin (IL)-2–producing Type 1 cells and IL-4–, IL-5–, and IL-13–secreting Type 2 cells, respectively.7 The Type 1-Type 2 dichotomy has been observed in a substantial number of infectious diseases and has frequently been associated with either susceptibility or resistance to the causative microorganisms.8 In addition, imbalances of Type 1 and Type 2 responses have been shown to contribute to the generation of allergic disorders.6

By use of flow cytometric analysis of cytokine-producing cells, we sought to characterize the phenotypes and frequencies of cytokine-producing T cells in healthy Africans compared with healthy Europeans. Both children and adults were included in order to give credit to the cumulative effect of encountered contacts with infectious agents.

PATIENTS AND METHODS

Study area. The African part of the study took place in the Albert Schweitzer Hospital in Lambarene, situated at the equator in the Gabonese rainforest. Previously conducted investigations have classified this region as predominantly hyperendemic for Plasmodium falciparum malaria; other malarial species, such as P. malariae and P. ovale, were much less encountered.7 Although not systematically studied yet, the wide range of other infectious diseases typical for Central Africa is found within the study area: bacterial diarrhea, respiratory infections, measles in childhood and other common viral diseases, tuberculosis, schistosomiasis, and intestinal helminths (unpublished observations). Fourteen adults (9 women, 5 men; median age, 31 years; age range, 20–63 years) and 8 children (4 girls, 4 boys; median age, 8 years; age range, 4–12 years), all apparently healthy and nonatopic Gabonese people or, in the case of children, their parents or guardians, were asked to participate when they met the following study criteria: no recent history of malaria or other systemic infections; no recent intake of any medication; normal presentation at physical examination, no clinical signs and symptoms; levels of the acute-phase reactant C-reactive protein below the level of detection (measured by immunonephelometry; Behring, Vienna, Austria). Carriers of Loa loa microfilariae have been shown to be associated with a more pronounced Type 2 response in comparison to microfilariae; therefore, such people were excluded from further analyses. The presence of malarial parasites was dis-
closed by the examination of Giemsa-stained thick blood smears; concurrent microfilaraemia was excluded by Giemsa-stained thick blood smears, leukoconcentration, and Nucleopore filtration methods.

The European part of the study was performed in Vienna, Austria. Age-matched healthy Austrians, and in the case of children, their parents, were asked to participate if they had no history of any systemic illness and atopy was ruled out (adults: 8 women, 7 men; median age, 33 years; age range, 22–54 years; children: 6 girls, 5 boys; median age, 7 years; age range, 2–13 years). Again, C-reactive protein levels had to be below the level of detection. In addition, patients who had recently traveled to sub-tropical or tropical countries were not included in the study. Fifteen adults and 11 children were included.

Patients or their parents provided informed consent, and the study was approved by the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambarene, Gabon.

Peripheral blood mononuclear cell cultures and intracellular detection of cytokines by flow cytometry. Flow cytometric assessment of T-cell cytokine production was performed essentially according to the technique described by Jung and others and modified by Willheim and others. Peripheral blood mononuclear cells were isolated from heparinized blood by Ficoll-diatrizoate centrifugation. Cells were then cultured in Ultra Culture Medium (Bio Whittaker, Walkersville, MD) supplemented with L-glutamine (2 mM; Sigma Chemical, St. Louis, MO), gentamicin (170 µg/L; Sigma), and 2-mercaptoethanol (3.5 µL/L; Merck, Darmstadt, Germany) and stimulated with phorbol 12-myristate 13-acetate (10 ng/mL; Sigma) and ionomycin (1.25 µM; Sigma) in the presence of monensin (1 µM; Sigma) for 4 hr at 37°C in 5% CO₂. Cells were then harvested on ice, washed twice in phosphate-buffered saline (PBS) and fixed with 2% formaldehyde (1 mL per 2 × 10⁶ cells; Merck) for 20 min. After 2 additional washes in PBS, cells were then resuspended in Hanks balanced salt solution (supplemented with 0.3% bovine serum albumin and 0.1% sodium-azide) and stored at 4°C in the dark until staining. Fixed cells were washed twice with PBS and made permeable with saponin (0.1%; Sigma), were resuspended with 50 µL of saponin-buffer–diluted antibodies, and were incubated for 25 min at room temperature in the dark. The following monoclonal antibodies were used: cytokine-specific mouse anti-human monoclonal antibody (mAb) IFN-γ [clone B27], fluorescein isothiocyanate (FITC) labeled and rat anti-human mAb IL-2 [Miq–17H12], phycoerythrin [PE] conjugated; IL-4 [MP4–25D2], PE labeled; IL-4 [MP4–25D2], FITC labeled; IL-5 [TRFK5], PE labeled; IL-13 [JES10–5A2], PE labeled). All mAbs were purchased from Pharmingen (San Diego, CA). The anti–CD4-mAb and anti–CD8-mAb were labeled with allopheyocyanin and peridinin, respectively (Becton Dickinson, Mountain View, CA). Four-color staining was performed, and at least 10⁶ cells were analyzed on a FACScanibur device (Becton Dickinson) equipped with a 2 laser system (488 nm and 630 nm wavelengths, respectively). All intracellular cytokines were stained in conjunction with CD4⁺ and CD8⁺. Data were analyzed with CELLQuest software (Becton Dickinson), and results were expressed as the percentage of cytokine-producing cells in each CD4⁺ or CD8⁺ cell population. Analyses on unstimulated cells have been performed, but staining results were always negative.

Statistical methods. Statistical analysis was performed by a standard statistical package (SPSS 7.5 for Windows; SPSS, Chicago, IL). Groups were compared by Kruskal-Wallis statistics, the Mann-Whitney U-test, and Dunn’s posttest comparison.

RESULTS

Percentages of CD4⁺ and CD8⁺ T cells, and CD4⁺/CD8⁺ peripheral blood mononuclear cells within the lymphocyte scatter gate. Both African and European children displayed a significantly higher percentage of CD4⁺/CD8⁺ mononuclear cells analyzed within the lymphocyte scatter gate than the respective adult groups (Table 1). Differences with regard to the CD4⁺ and CD8⁺ subsets were not significant between groups.

Frequency of Type 1 cytokine-producing CD4⁺ and CD8⁺ T cells in Africans and Europeans. Ex vivo IL-2 and IFN-γ production, and cytoplasmic coexpression of both cy-

### Table 1

<table>
<thead>
<tr>
<th>Study group</th>
<th>Median % of CD4⁺ Median % of CD8⁺</th>
<th>Median % of CD4⁺</th>
<th>CD8⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>European children</td>
<td>45 (35–63)</td>
<td>18 (13–29)</td>
<td>37 (27–46) *</td>
</tr>
<tr>
<td>African children</td>
<td>48 (33–60)</td>
<td>15 (12–28)</td>
<td>37 (24–41) †</td>
</tr>
<tr>
<td>European adults</td>
<td>50 (40–55)</td>
<td>25 (18–29)</td>
<td>25 (23–41)</td>
</tr>
<tr>
<td>African adults</td>
<td>54 (40–63)</td>
<td>22 (16–30)</td>
<td>24 (20–38)</td>
</tr>
</tbody>
</table>

* P < 0.01 compared with European adults.
† P < 0.01 compared with African adults.

### Table 2

<table>
<thead>
<tr>
<th>Frequency of type 1 cytokines producing T cells in study populations</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2⁺/IFN-γ⁺</td>
<td>34.4 ± 2.4</td>
<td>32.7 ± 1.7</td>
</tr>
<tr>
<td>IL-2⁺/IFN-γ⁺⁺</td>
<td>7.7 ± 1.2</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>IL-2⁺/IFN-γ⁺⁻</td>
<td>2.2 ± 0.3</td>
<td>5.7 ± 0.8⁻</td>
</tr>
<tr>
<td>IL-2⁺/IFN-γ⁻⁻</td>
<td>6.1 ± 0.7</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>IL-2⁺/IFN-γ⁻⁻⁻</td>
<td>6.6 ± 1.2</td>
<td>3.6 ± 0.4⁻</td>
</tr>
</tbody>
</table>

Note: CD4⁺ = IL-2⁺/IFN-γ⁺⁺⁺; P < 0.01; IL-2⁺/IFN-γ⁺⁺⁺ = P < 0.001; P < 0.001; IL-2⁺/IFN-γ⁺⁺⁺ = P < 0.01; P < 0.001; IL-2⁺/IFN-γ⁺⁺⁺ = P < 0.01; IL-2⁺/IFN-γ⁺⁺⁺ = P < 0.01; IL-2⁺/IFN-γ⁺⁺⁺ = P < 0.01; IL-2⁺/IFN-γ⁺⁺⁺ = P < 0.01; IL-2⁺/IFN-γ⁺⁺⁺ = P < 0.01. Data are presented as percentages ± SEM. IFN = interferon; IL = interleukin.
* Significant differences between African children and adults.
† Significant differences between European children and adults.
‡ Significant differences between European and African adults.
§ Significant differences between African children and adults.
tokines within CD4<sup>+</sup> and CD8<sup>+</sup> T cells were studied; results are depicted in Table 2. The overall frequency of IL-2– and IFN-γ–producing T cells within the 4 study groups is shown in Figure 1. The most striking finding was the marked increased capacity of IFN-γ production in both CD4<sup>+</sup> and CD8<sup>+</sup> T cells and of IL-2 in CD4<sup>+</sup> T cells of adult Africans when compared with their age-matched European counterparts. In addition, age-dependent increases of Type 1 cytokine-producing T cells were more evident within the African study population (Figure 1).

**Frequency of Type 2 cytokine-producing CD4<sup>+</sup> and CD8<sup>+</sup> T cells in Africans and Europeans.** Type 2 cytokine-expressing T cells were more frequent in African people; however, significant differences were shown only between the adult groups (Table 3, Figure 2). A tendency towards higher frequencies of IL-4– and IL-13–producing CD4<sup>+</sup> and CD8<sup>+</sup> T cells were found in African children; however, differences between those children and European children did not reach statistical significance. In all, expression of IL-4 was strongly correlated with that of IL-13, except for CD8<sup>+</sup> cells in African adults, who were substantial producers of IL-4, but not of IL-13. With the exception of IL-13 expression in the CD8<sup>+</sup> subset, Type 2 cytokines were more frequently expressed in African adults than in African children (Figure 2).

**Frequency of CD4<sup>+</sup> and CD8<sup>+</sup> T cells producing a Type 0 pattern of cytokines in Africans and Europeans.** Both Type 1 and Type 2 cytokines producing T cells were more frequent in Africans, especially in adults, and not surprisingly, this was also true for T cells coexpressing IFN-γ with IL-4 (Table 3). The frequency of IL-13/IFN-γ–expressing T cells was generally low within all study groups.
FIGURE 2. Overall frequency of T cells expressing a Type 2 (interleukin [IL]-4, IL-13) and a Type 0 pattern (interferon [IFN]-γ/IL-4, IFN-γ/IL-13) of cytokines in children (AC) and adults (AA) from Central Africa and Central Europe (EC and EA). * Significant differences between African children and adults. † Significant differences between African and European adults. Differences between European children and adults as well as between African and European children were not significant. The line through the box shows the 50% of values (median), with the other quartiles at either end. Values that varied by more than 1.5 times the length of the box are not depicted. CD4+IL-4*: P < 0.01; † P < 0.001; CD8+IL-4+: * P < 0.05, † P < 0.01; CD4+IL-13+: * P < 0.05, † P < 0.01; CD8+IL-13+: differences not significant.

TABLE 3
Frequency of type 2 and type 0 cytokines producing T cells in study populations

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>CD4</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>European children</td>
<td>African children</td>
</tr>
<tr>
<td>IL-4+/IFN-γ−</td>
<td>2.6 ± 0.5</td>
<td>4 ± 0.6</td>
</tr>
<tr>
<td>IL-4+/IFN-γ+</td>
<td>1.3 ± 0.4</td>
<td>2.1 ± 0.3§</td>
</tr>
<tr>
<td>IL-13+/IFN-γ−</td>
<td>2.5 ± 0.4</td>
<td>3.8 ± 0.6</td>
</tr>
<tr>
<td>IL-13+/IFN-γ+</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

* Significant differences between African children and adults.
† Significant differences between European and African children.
§ Significant differences between European children and adults.

Note: CD4+ IL-4+/IFN-γ−: * P < 0.05; † P < 0.001; IL-4+/IFN-γ+: * P < 0.05; † P < 0.001; IL-13+/IFN-γ−: * P < 0.05, † P < 0.01; IL-13+/IFN-γ+: * P < 0.05, † P < 0.001. CD8+ IL-4+/IFN-γ−: * P < 0.05; † P < 0.001; IL-4+/IFN-γ+: † P < 0.001; IL-13+/IFN-γ−: † P < 0.01. Data are presented as percentages ± SEM. IFN = interferon; IL = interleukin.
DISCUSSION

The findings indicate a strikingly increased frequency of cytokine-producing T cells in African adults when compared with their age-matched European counterparts. Differences were shown for both Type 1 and Type 2 cytokines, as well as for cytokines produced following a Type 0 pattern. A trend toward an increased frequency of cytokine-producing CD4+ and CD8+ cells was even shown for African compared with European children; however, this increase reached significance only for the exclusively IFN-γ-producing CD4+ T-cell subset. An age-dependent increase in the overall capacity of cytokine production was evident within the African population; the significant increased expression of intracytoplasmic cytokines in European adults was restricted to IFN-γ within the CD4+ subset and to IL-2 within the CD8+ subset.

The latter is in line with the results of a Japanese study, which revealed increases in Type 1 cytokine production in both CD4+ and CD8+ T cells. Of special interest was the fact that the Japanese adults displayed about the same frequency of cytokine-expressing cells as the European people studied here. This suggests that the cumulative experiences of antigenic contacts rather than ethnic differences are determinants for T-cell cytokine generation. Further support for this hypothesis is derived from a recent Brazilian study of people infected with *Wuchereria bancrofti*, which identified these people as having an increased frequency of IFN-γ- and IL-4–producing CD4+ cells when compared with North American controls.

In our study, with the exception of exclusively IFN-γ–producing CD4+ cells, significant differences between Africans and Europeans were found only in adults. Therefore, we propose that the quantity of the age-associated increase in T-cell cytokine production is largely dependent on the presence or absence of a continuous antigenic challenge. It can be speculated that the moderate age-dependent increase in the frequency of Type 1 cytokine-producing T cells observed in Europeans and Japanese is due to a rather limited expansion of primed cells, most probably of a memory phenotype, and is induced by viral agents or vaccines. The exclusive induction of a Type 1 cytokine response in lymphocytes during measles has recently been reported. Common parasites such as helminths or even malaria that preferentially initiate a Type 2–biased immune response, as recently pointed out, are virtually absent in Europe and Japan. Thus, it is not surprising that the frequency of IL-4– and IL-13–expressing T cells remains at a low level throughout life in nonatopic people from these regions.

For the reasons mentioned above, we feel that genetic differences between Africans and Europeans are unlikely to substantially account for the profound differences in cytokine production profiles in this study. However, we cannot exclude at least some contribution of the genetic background to the observed differences, especially in the light of recently discovered polymorphisms within promoter regions of genes encoding for several cytokines (unpublished results). The impact of frequently encountered infections on immune responsiveness has not been studied so far in healthy people. Because both CD4+ and CD8+ cells display major immunoregulatory and effecter roles in adaptive immunity, it appears plausible that the pronounced differences in the frequency of cytokine-producing T cells between Africans and Europeans are of substantial clinical significance.

From a methodological point of view, the findings are of particular interest because the increased capacity of cytokine production in Africans has been elaborated by use of non-specific mitogens, thus avoiding the necessity to restrict the observed differences to a specific antigen. This further implies that the development of vaccines—irrespective of the antigens targeted—has to be adapted to the specific regional pattern of immune responsiveness. In this context, we refer to the conflicting results of recent malaria vaccine trials, which obviously have been conducted without this premise. Whether the observed age-dependent differences in Type 1 T-cell cytokine production contribute to more severe manifestations of common viral diseases such as measles in adults remains to be evaluated.

It is intriguing to speculate that the decreased expression of Type 2 cytokines in Europeans when compared with Africans has an impact on the continuously increasing prevalence of allergic disorders in Europe in contrast to tropical Africa. Functionally, this may be due to the dominance of a parasite-associated Type 2 response in Africans that, by producing large quantities of polyclonal immunoglobulin E, induces the saturation of immunoglobulin E binding sites on mast cells, thereby inhibiting the sensitization to environmental allergens.

Obviously, at present, the clinical consequences that result from the findings of our study need to be addressed in large longitudinal studies with special reference to genetic and environmental factors. Yet we propose that the regional differences in immune reactivity, as we have shown in this study, should strongly be considered in all aspects dealing with immune responsiveness and clinical immune intervention strategies.

Acknowledgments: We thank Heidi Winkler, Marcel Nkeyi, and Anselme Nzengue for their help. The study was in part supported by a grant from the Austrian Society of Chemotherapy, from the Medizinisch-Wissenschaftlicher Fonds des Bürgermeisters der Bundes-Hauptstadt Wien (project 1516), from the fortune programme, Medical Faculty, University of Tübingen, and the EU-INCO-DC programme.

Authors’ addresses: Astrid Wilfing, Stefan Winkler, Alexander Aichburg, and Wolfgang Graninger, Department of Internal Medicine I, Division of Infectious Diseases, University of Vienna, Währingergürtel 18-20, A-1090 Vienna, Austria. Stefan Winkler, Alexander Aichburg, and Peter G. Kremsner, Research Unit of the Albert Schweitzer Hospital, Lambaréné, B.P. 118, Gabon. Karl Schrattbauer, Martin Willheim, and Karin Baier, Institute of General and Experimental Pathology, University of Vienna, Währingergürtel 18-20, A-1090 Vienna, Austria. Thomas Müller, Department of Pediatrics, University of Vienna, Währingergürtel 18-20, A-1090 Vienna, Austria. Peter G. Kremsner, Department of Parasitology, Institute of Tropical Medicine, University of Tübingen, Wilhelmstrasse 27, D-72074 Tübingen, Germany.

Reprint requests: Winkler Stefan, Department of Internal Medicine I, Division of Infectious Diseases, University of Vienna, Währingergürtel 18-20, A-1090 Vienna, Austria, Telephone: 0043-1-40400-4440, Fax: 0043-1-40400-4418 (e-mail: stefan.winkler@akwien.ac.at).
REFERENCES


