Immune dysregulation in Ethiopian immigrants in Israel: relevance to helminth infections?

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(Accepted for publication 3 October 1995)

SUMMARY
The infectious disease background and particularly the helminth infections that are endemic in Africa could have profound effects on the host immune system. Studies that we have performed on an Ethiopian HIV+ immigrant population that has recently reached Israel, lend support to this notion. They have indeed revealed a very high prevalence of helminth and several other infections with an extreme immune dysregulation, consisting of: (i) highly elevated plasma IgE, IgG, placental isoferitin, p75 soluble TNF receptor (sTNFR) levels and very high blood eosinophilia; (ii) increased secretion from phytohaemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) of the cytokines IL-2, IL-4, IL-10 and p75 sTNFR, and decreased secretion of interferon-gamma (IFN-γ) and IL-6; (iii) increased and decreased surface expression of p75 TNFR and IL-6 receptor on lymphocytes, respectively. The causal relationship between this immune dysregulation and the infectious background is highly suggestive, and could have far-reaching implications in the resistance to other infections.

Keywords: cytokines, immune dysregulation, T helper cell subsets, helminth infections, Ethiopian immigrants

INTRODUCTION
One of the prominent features of the health situation in Africa and other developing countries is the high endemic prevalence of chronic infectious diseases [1]. The host confronted with the infectious burden would be expected to mount prolonged immune response to this challenge which would alter the normal immune balance. Such changes could also have profound effects on the ability to cope with other infections and could also affect the general and specific immune response to other stimuli.

A striking characteristic of the AIDS epidemic in Africa is the different pattern of the disease with its mode of transmission, rapid spread and fast progression [2-7]. The reasons for this different pattern are not clear. We have recently suggested that changes in the host immune response caused by endemic infections and mostly helminth infections could account for at least a part of this pattern [8].

This idea has been supported by our experience and studies of an Ethiopian immigrant population that has recently reached Israel. They have indeed revealed a very high prevalence of many infections (helminth infections being almost universal), as well as a high prevalence of HIV-1. Furthermore, an extreme degree of immune dysregulation was observed in this population, independently of the HIV infection status. These findings have prompted this study.

SUBJECTS AND METHODS

Human subjects
Two groups of clinically healthy HIV+ individuals were studied: (i) Ethiopian immigrants, shortly after their arrival in Israel; and (ii) non-African Israelis, matched for age and sex.

Immunoglobulins and placental isoferitin levels
Plasma IgG and IgE were determined by routine nephelometry (Beckman IgG; Beckman Instruments, Inc., Galway, Ireland) and fluorometry (Delfia Total IgE Kit; Wallace Oy, Turku, Finland) techniques. Serum placental isoferitin (PLF) was determined by ELISA as described [9]. Briefly, CM-G-8 MoAb was coupled to a 96-well Maxisorp Nunc Immunoplates (Nunc, Roskilde, Denmark) and test sera were added to the wells. After incubation and washing, alkaline phosphatase (Sigma, St Louis, MO)-conjugated CM-h-9 MoAb was added to each well. Colour was developed by the addition of p-nitrophenyl-phosphate substrate (Sigma), and read in an ELISA...
reader at 405 nm. The amount of PLF that bound 250 μg of alkaline phosphatase-conjugated CM-h-9 MoAb was arbitrarily considered to be 10 U of PLF, and the results were expressed as U/ml.

Cell culture supernatant preparation

Peripheral blood mononuclear cells (PBMC) were obtained from heparinized venous blood by standard centrifugation over Lymphoprep (Nycomed Pharma As, Oslo, Norway), washed and resuspended at 2 x 10⁶/ml in RPMI 1640 (Biological Industries Co. Bet-Haemek, Israel) supplemented with 5% heat-inactivated human AB serum (Sigma), 2 mM l-glutamine and antibiotics—penicillin, streptomycin and nystatin (Biological Industries). Cells (1 ml/well) were cultured in 24-well Multidish plates (Nunc) at 37°C under 6.5% CO₂ with and without phytohaemagglutinin (PHA: 1:100) (Difco PHA-P; Detroit, MI) for 72 h. For IL-2 production, PBMC (1 x 10⁶/well) were seeded in 96-well flat-bottomed plates (Nunc) in RPMI 1640 medium for 1 h at 37°C, 6.5% CO₂. Subsequently, PHA (1:100) and human anti-IL-2 receptor antibody, anti-Tac (2.5 μg/ml) (kindly provided by Hoffman-La Roche Inc., Nutley, NJ) to prevent IL-2 consumption by the stimulated cells, were added and the cells were cultured for 6 days at 37°C, 6.5% CO₂ [10]. All supernatants (SN) were collected by centrifugation and stored at −70°C until tested.

Cytokine and cytokine receptor determination

IL-2 activity in SN was assessed as the ability to stimulate the proliferation of the IL-2-dependent mouse cell line, CTLL, as described [10]. Briefly, 1 x 10⁵ CTLL/well and three-fold successive dilutions of SN were cultured for 24 h, pulsed with 1 μCi of ³H-thymidine (Rotem Industries Ltd, Nuclear Research Center-Negev, Israel) and harvested 18 h later. Results are expressed as mean cpm for three replicate wells for a given SN dilution.

For determination of IL-6 activity in plasma, B9 cells were used in a standard assay as described [11]. Briefly, serial dilutions of heat-inactivated plasma samples and recombinant Escherichia coli-derived IL-6 (Amersham, Aylesbury, UK) were distributed to 96-well flat-bottomed plates (Nunc). Five thousand B9 cells in 0.1 ml medium containing 10% RPMI 1640, 10% fetal calf serum (FCS; Biological Industries), 2 μM l-glutamine and antibiotics, were added into each well. Plates were incubated for 72 h at 37°C under 6.5% CO₂ and pulsed with ³H-thymidine for the last 18 h. Data are expressed as U/ml calculated from the standard curve of rat IL-6.

For determination of IL-6 activity in SN, 35,000 T-1165 cells [12] were cultured with serial dilutions of the SN and human rIL-6 (15 x 10⁶ reference units/mg protein) (Interpharm, Ares-Serono Group, Nes-Ziona, Israel) in 96-well flat-bottomed plates (Nunc) in 0.1 ml of RPMI 1640 culture medium containing 10% FCS, 4 μM l-glutamine, 2 x 10⁻³ M 2-mercaptoethanol and antibiotics. After an incubation period of 48 h at 37°C in 6.5% CO₂, the wells were exposed to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) for 2 h at 37°C as described [13]. Subsequently, 0.1 ml solution of 20% SDS (Sigma) 1:1 water:N,N dimethylformamide (Sigma), pH 4.7, was added overnight. Absorption was measured at 450 nm and the data were calculated from the standard curve of human rIL-6.

Interferon-gamma (IFN-γ), IL-4 and IL-10 levels in SN were determined by ELISA using CytoScreen Immunossay Kits (BioSource Int., Camarillo, CA) according to the manufacturer's instructions.

Plasma levels of sIL-6r and soluble p75 tumour necrosis factor receptor (sTNFR) as well as p75 TNFR levels in SN were determined by specific ELISA according to reported procedures [14-15]. Briefly, MoAbs to sIL-6r [14] or to p75 TNFR[16] were absorbed to 96-well Maxisorp Nunc Immulon plates. After 2 h incubation at 37°C, the wells were blocked with 1% bovine serum albumin (BSA) for an additional 2 h, washed and rabbit polyclonal antisera to the sIL-6R or to the p75 TNFR were added to the wells. After overnight incubation at 4°C the plates were washed and incubated for 2 h with horseradish peroxidase-conjugated purified goat-anti-rabbit IgG (Jackson Labs, West Grove, PA). The assay was developed using ABTS (Sigma) as a substrate. The enzymatic product was determined colorimetrically at 405 nm. Purified sIL-6R [14] and p75 TNFR[16] MoAbs, or with matched MoAbs (Becton Dickinson, San Jose, CA) served as a background isotype negative control. After staining, cells were washed twice and then incubated at 4°C for 30 min with FITC-conjugated goat-anti-mouse F(ab)² fragment of affinity-purified antibodies to Fab fragment of mouse IgG (BioMakor, Rehovot, Israel). Cells were washed and analysed by flow cytometry with a FACScan (Becton Dickinson). The percentage of membrane fluorescent cells were acquired on a log scale (5000 events per sample) on gated lymphocytes defined in forward versus side scattering.

Statistical analysis

Comparisons between groups were performed by the Wilcoxon two-sample test, using the Epistat software (Epistat Services, Richardson, TX).

RESULTS

Immune activation markers in the blood

Several immune activation markers were determined in the blood of the studied population and are depicted in Fig. 1. As can be seen, plasma levels of IgG, IgE, and serum levels of PLF were significantly higher in the Ethiopian population in comparison with the non-Ethiopian control group (P < 0.001-0.01). Most strikingly, the mean plasma levels of IgE among the Ethiopian immigrants reached values of over five-fold more than mean levels among the non-Ethiopian controls, the means ± s.e.m. being 753 ± 123 U/ml and 143 ± 33 U/ml, respectively. Interestingly, it should also be noted that such extreme elevations of plasma IgE among the Ethiopians were not accompanied by any allergic manifestations. The difference in serum levels of PLF between the groups was even more pronounced, the means ± s.e.m. being

1996 Blackwell Science Ltd, Clinical and Experimental Immunology, 103:239-243
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10-fold more in the Ethiopians compared with non-Ethiopians, 52 ± 38 U/ml and 5 ± 9 U/ml, respectively. Blood eosinophil counts were above the normal range in half of the Ethiopian studied population (not shown). Cytokine studies revealed significantly elevated levels of plasma p75 sTNFR (P < 0.01), the mean ± s.e.m. being 4.6 ± 0.3 ng/ml and 3.5 ± 0.2 ng/ml in the two groups, respectively, while the differences in plasma levels of IL-6 and of sIL-6R were not significant. Plasma levels of several other cytokines—IL-2, IL-4, IL-10, and IFN-γ—were found to be undetectable or unquantifiable, and thus these cytokines were measured by their secretion into SN of PBMC.

Cytokine secretion by PBMC

Secretion of several cytokines by PBMC was elevated in PBMC obtained from Ethiopians in comparison with non-Ethiopian controls. The results of these studies are depicted in Fig. 2. Of the Th2 type cytokines, secretion of IL-4 and IL-10 was significantly elevated, 231 ± 64 pg/ml versus 64 ± 13 pg/ml and 2115 ± 286 pg/ml versus 1141 ± 127 pg/ml (mean ± s.e.m.), respectively, while that of IL-6 was decreased in the Ethiopians, 1143 ± 182 U/ml versus 2369 ± 372 U/ml.

Of the Th1 type of cytokines, secretion of IL-2 was elevated in the Ethiopians compared with non-Ethiopians, 55169 ± 931 ct/min, 30720 ± 986 ct/min, respectively, while the secretion of IFN-γ was significantly decreased in the Ethiopians compared with non-Ethiopians, being 2136 ± 106 pg/ml versus 3706 ± 160 pg/ml, respectively.

Secretion of p75 sTNFR, representing the TNF system and therefore not clearly defined in terms of Th1 or Th2 type cytokines, was also clearly elevated in the Ethiopians compared with non-Ethiopians, the mean ± s.e.m. being 14 ± 4 ng/ml compared with 0.25 ± 0.2 ng/ml, respectively.

Cytokine membrane expression

Expression of cytokine receptors on lymphocyte membranes was studied for IL-6R and for TNFR. The two receptors were found to behave differently, the percentage of lymphocytes expressing p75 TNFR being increased while that of

![Graphs showing cytokine levels and secretion](image-url)
lymphocytes expressing IL-6R was decreased, in Ethiopians compared with non-Ethiopians: 20.9% versus 19.7% (P < 0.01), and 24.7% versus 36% (P < 0.01), respectively (Fig. 3). The increased expression of p75 TNFR was strongly correlated to the increased secretion of this molecule by PBMC (Fig. 2). The decreased expression of IL-6R is probably related to the decreased secretion of the IL-6 (Fig. 2), though whether this is a causal relationship remains to be determined.

**DISCUSSION**

The results of these studies have demonstrated that a broad type of immune dysregulation is found in HIV− new immigrants from Ethiopia independent of HIV infection. It consisted of elevated plasma IgE and IgG, blood eosinophilia, and increased serum PLF and p75 TNFR. Other studies revealed elevated secretion of IL-2, IL-4, IL-10, and sTNFR, and decreased secretion of IFN-γ and IL-6 from PHA-stimulated PBMC. Furthermore, expression of cytokine receptors on lymphocyte membranes was found to be decreased for IL-6R, while being increased for p75 TNFR.

The presence of such broad immune dysregulation in our study group of Ethiopian new immigrants is in all likelihood representative of a general phenomenon in the wide population of Ethiopia and other countries in Africa. It is probably present in other developing countries as well, and our recent study of a small population of Thai laborers in Israel that has revealed similar findings, indeed lends support to this expectation [18]. The most likely and simple explanation for such wide immune disturbances is the very high prevalence of infectious and parasitic diseases endemic in these areas. This is certainly the case for the Ethiopian new immigrants that we had the opportunity to study upon their arrival in Israel. As can be seen in Table 1, this population is highly infected by various types of infections. Note should be taken also of the relatively high percentage of HIV− carriers in this population, which is over 100 times the prevalence of HIV− infection in the Israeli non-Ethiopian population.

A central question in the African scenario is whether there is a specific influence for certain infections on the immune profile. One of the striking elements in the infectious load in Africa and other developing countries are the helminth infections, which are universal and chronic lifetime infections [20,21]. As shown above, such infections were very common among the Ethiopians that we studied. Although our understanding of the mechanisms involved in the handling of helminth infections is limited, they result in eosinophilia, high levels of IgE and mast cell activation, which are suggestive for increased IL-4 and IL-5 secretion [22]. Such influences could then serve as a major element in shifting the balance toward a Th2 type of immune response.

Is the immune activation we have observed in the HIV non-infected Ethiopian immigrants a Th2 type of activation? The answer to this question is not simple. The Ethiopians had elevated plasma IgE, IgG and eosinophilia, and had increased secretion of IL-4 and IL-10 from their PBMC, all of which would fit with a Th2 type of response. Furthermore, they also had decreased secretion of IFN-γ, indicating a lowered Th1 response. On the other hand, the decreased secretion of IL-6, and the increased secretion of IL-2 by their PBMC, would fit better with a Th1 type of immune response. Thus, our findings clearly indicate a mixed profile of Th1 and Th2, and may reflect the true in vivo state where the prevalence of Th0 cells is more dominant [23,24]. Additional factors that may play a role in our system and can explain the results are: (i) the activated TNF system, probably as a result of the helminth infections [22], may affect the cytokine profile [25,26]; (ii) the presence of elevated IL-4 does not inhibit the proliferation of Th1 cells secreting IL-2 [25]; (iii) the stimulation of memory cells by other infections is very likely to be continually present, and would increase at least the IL-2 secretion in vitro by these proliferating cells.

The activation of the PLF has not yet been categorized to one of the Th types. As a molecule with a physiological immunosuppressive function, it is of great interest that increased levels of this molecule are also found during HIV infection and may be correlated with the stages of the infection [26]. Activated CD4+ T-cells are a major source and, thus, the extremely high serum levels of PLF found in the HIV+ Ethiopian population indicate a high degree of CD4+ cell activation, accompanied by a certain degree of immunosuppression [27].

Table 1. Rates of some infections in Ethiopian immigrants [%]*

<table>
<thead>
<tr>
<th>Infection</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Enterobacter cloacae</td>
<td>24</td>
</tr>
<tr>
<td>Vector American</td>
<td>50</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>79</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>38</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
<td>19</td>
</tr>
<tr>
<td>Plasmodium vivax</td>
<td>10</td>
</tr>
<tr>
<td>Hepatitis B virus carriers</td>
<td>12</td>
</tr>
<tr>
<td>Hepatitis B virus-exposed</td>
<td>18</td>
</tr>
<tr>
<td>Trypanosoma rhodesi</td>
<td>31</td>
</tr>
<tr>
<td>HIV-1</td>
<td>2</td>
</tr>
</tbody>
</table>

* Data (in round figures) are from [18] and from our own unpublished observations.
Our findings in the Ethiopian immigrants may be unified under the less restrictive general heading of immune activation. Such activation, with the helminth infection in its background, may serve to facilitate and influence the course of other infections, including HIV [28–31]. The orchestration of the immune network is dependent on a continuous balance among individual cytokines. This accounts for the sometimes polar outcomes of the response with the same ‘players’. We believe that this also holds true for the scene in Africa and the developing world where immune activation is continuous and the result of chronic persistent stimulation [8,32]. The implications of these findings are manifold. On the level of public health and preventive measures in Africa and other developing countries, a much greater emphasis on the eradication of helminth infections should be given priority. Such eradication may modulate the baseline immune response and lead to improved ability to cope with infections. On the level of protective vaccine development, it is to be expected that the type of immune response and success of vaccines would be very different in Africa than in the developed world, and therefore different approaches will have to be used there.

ACKNOWLEDGMENTS

We thank I. Cohen and G. Birke for critically reviewing the manuscript. D. Wallach and D. Novick for their generous gifts of the components needed for TNFR and IL-6 determinations, and Hoffman-La Roche Inc., Nutley, NJ for the generous gift of anti-Tac for the determination of IL-2 secretion. The technical assistance of N. Harpaz, E. Perah, L. Horenstein and B. Zingerman is gratefully acknowledged. This work was supported by grants from the Israel Ministry of Health.

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