**Chronic immune activation associated with intestinal helminth infections results in impaired signal transduction and anergy**

Gadi Borkow,1 Qibin Leng,1 Ziva Weisman,1 Miguel Stein,1 Noya Galai,2 Alexander Kalinkovich,1 and Zvi Bentwich1

1R. Ben-Ari Institute of Clinical Immunology and AIDS Center, Kaplan Medical Center, Hebrew University Hadassah Medical School, Rehovot, Israel
2Department of Epidemiology, Ben-Gurion University, Beer-Sheba, Israel

Address correspondence to: Zvi Bentwich, R. Ben-Ari Institute of Clinical Immunology, AIDS Center, Kaplan Medical Center, Rehovot 76100, Israel. Phone: 972-8-9441444; Fax: 972-8-9410461; E-mail: bentwich@agri.huji.ac.il.

Gadi Borkow and Qibin Leng contributed equally to this work.

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Helminthic parasites cause widespread, persistent infections in humans. The immigration of Ethiopians to Israel (a group denoted here by “Eth.”), many of them infested with helminths and in a chronic immune-activation state, enabled us to investigate the effects of such immune activation on immune responses. We studied the immune profile and immune functions of 190 Eth. and Israeli non-Eth. (Isr.) highly, partially, or non–immune-activated individuals. Immune cells from highly immune-activated individuals were defective in several signaling responses, all of which were restored gradually following anti-helminthic treatment. These cells showed poor transmembrane signaling, as seen by the phosphorylation of various tyrosine kinases and of the MAPK kinases, ERK1/2 and p38; deficient degradation of phosphorylated IκBα; increased expression of cytotoxic T lymphocyte–associated antigen 4 (CTLA-4), which appears to block proliferative responses in these cells; decreased β-chemokine secretion by CD8+ cells after stimulation; and reduced proliferation to recall antigen stimulation. Highly immune-activated individuals also showed decreased delayed-type skin hypersensitivity responses to recall antigen before deworming. These findings support the notion that chronic helminthic infections cause persistent immune activation that results in hyporesponsiveness and anergy. Such impaired immune functions may diminish the capacity of these individuals to cope with infections and to generate cellular protective immunity after vaccination.


**Introduction**

Helminthic infections are common in vast regions of the world, especially in the developing countries, and by the most conservative estimates, they affect more than a quarter of the world’s population (approximately 1.5 billion people) (1). In addition, millions of individuals in the developing countries also have other chronic infectious diseases, such as malaria, tuberculosis (TB), protozoa, and HIV (2, 3). These infections lead to persistent activation of the immune system, and thus vast populations in the globe, especially in Africa and Asia, are in a chronic immune-activation state. However, how chronic immune activation affects the capacity of the immune system to respond to stimuli is not well understood.

During the last 15 years, about 60,000 Ethiopian Jews emigrated from Ethiopia to Israel. A very high prevalence of several infectious diseases, particularly helminthic infections, has been found among the population of Ethiopian immigrants to Israel (denoted here by “Eth.”) (4). More than 80% of the Eth. individuals were infected with at least one helminthic parasite, 40% were infected with two parasites, and some (~3%) were even infected with four intestinal helminthic parasites. Although Eth. individuals who immigrated 10–15 years ago were not infected with HIV, almost 10% of the Eth. individuals who immigrated during the last year were HIV-1 seropositive (data not shown). All Eth. individuals arrived from areas in which TB is endemic; however, the prevalence of TB was relatively low (<1.5%) (4).

Examination of the immune profile of the HIV-seronegative, *Mycobacterium tuberculosis*-noninfected members of the Eth. group revealed a wide immune dysregulation characterized by a dominant Th2 type of cytokine immune response, high IgE levels, eosinophilia, low CD4+ and high CD8+ cells, increased HLA-DR–positive cells in all examined T-cell subsets, decreased proportion of naive CD4+ cells, and increased proportion of CD4+ memory cells (5–7). This immune dysregulation, caused mainly by helminthic infections (6, 7), is assumed to be widespread in Ethiopia and in other parts of Africa where helminthic infections are endemic. Indeed, similar immune profiles were
observed in other populations from Ethiopia (8) and other developing countries (9–13). After their arrival in Israel, most Eth. individuals were cured of the helminthi-
cic infections (ref. 14; data not shown) and their immune systems slowly returned to normal (7, 15).

In the present report, we studied the immune-activa-
tion status and the capacity of PBMCs to respond to various stimuli in three groups: recently arrived Eth. individuals (new-Eth.) with helminthic infection after anti-helminthic treatment, and healthy noninfected Israeli-born (Isr.) individuals. In the study, we deter-
mimed (a) expression of costimulatory and downregu-
latory receptors on T cells (CD28 and cytotoxic T lymphocyte–associated antigen 4 [CTLA-4], respectively); (b) intracellular events that occur immediately after T-
cell stimulation (signal transduction); (c) long-term outcome events, such as proliferation after recall anti-
gen stimulation, and β-chemokine secretion by CD8+ cells; and (d) delayed-type skin hypersensitivity (DTH) responses to recall antigens.

The results of this study clearly show that chronic immune activation results in hyporesponsiveness, manifested by impaired T-cell signal transduction, sig-
nificantly lower expression of CD28 with concomitant increased expression of CTLA-4, decreased β-
chemokine secretion, low proliferation to recall anti-
gen, and decreased DTH responses.

Methods

Human subjects. A total of 151 Eth. and 31 Isr. individu-
als were studied; 111 of the Eth. subjects had been in
Israel for fewer than 12 months and did not receive anti-helminthic treatment at the time of their exami-
nation. More than 80% of them were heavily infested by
one or more helminthic parasites, as determined by
stool examination. Forty-eight Eth. subjects had been
living in Israel 3–10 years, and although they received
anti-helminthic treatment, in the stool of about 15% of
them, we found a few eggs of Schistosoma mansoni or
Trichuris trichuria. All Isr. subjects were free of helminthi-
cic parasites, and all individuals were HIV-seronegative
as determined by ELISA (Abbott Gmb Diagnostika,
Weisbaden, Delkenheim, Germany).

Cell preparations and cultures. PBMCs, isolated from
heparinized blood by standard centrifugation over
Histopaque (Sigma, Rehovot, Israel), washed and
resuspended in RPMI 1640 medium supplemented with
10% heat-inactivated human AB serum (Sigma),
2 mM L-glutamine, penicillin, streptomycin, and nys-
tatin (Biological Industries Co., Beit-Haemek, Israel),
were cultured at 37°C under 5% CO2. PBMCs (10^6
cells/200 μl), cultured in 96-well plates (Corning-
Costar, Corning, New York, USA) for 6 days in the
presence of 5 μg/ml tuberculin purified protein
derivative (PPD) (Statens Serum Institute, Copen-
hagen, Denmark), with or without 0.66 μg/ml
anti–CTLA-4 Fab mAb (Serotec Ltd., Oxford, United
Kingdom), were pulsed with 1 μCi [3H]thymidine
(Amersham Pharmacia Biotech, Little Chalfont,
Buckinghamshire, United Kingdom). Sixteen hours
later, the cells were harvested on Whatman 934-AH
glass microfiber filters (Whatman International,
Maidstone, United Kingdom), and the radioactivity
was measured by a Packard Tri-Carb 300 β-counter
(Packard Instrument Co., Meriden, Connecticut,
USA). Cells cultured in the presence of medium only
served as control. All experiments were done in trip-
licate, and the results are expressed as stimulation
index (SI; mean cpm obtained with PPD stimula-
tion/mean cpm obtained for control). The CTLA Fab
mAb’s were prepared from IgG mAb by papain diges-
tion and separation of the Fab fragments with a Cen-
tricon concentrator (Amicon Inc., Beverly, Massa-
echusetts, USA). The effectiveness of digestion was
verified by SDS gel electrophoresis. To measure the
production of chemokines, CD8+ cells (3 × 10^6) were
separated from PBMCs (~10 × 10^6) by positive selec-
tion using anti-CD8 immunomagnetic beads and
miniMACS columns according to the manufacturer’s
instructions (Miltenyi Biotec GmbH, Beryisch-Glad-
bach, Germany). Purity of enriched CD8+ cells was
greater than 95%, as determined by flow cytometry.
The cells were stimulated with 1 μg/ml PHA (Difco
Laboratories, Detroit, Michigan, USA) and 20 ng/ml
PMA (Sigma) for 48 hours. ELISA kits for human
MIP-1α and RANTES were used for determining chemokine levels in the supernatants according to
manufacturer’s instructions (R&D Systems, Min-
neapolis, Minnesota, USA).

Lymphocyte phenotype analysis. One- and two- or
triple-color immunophenotyping of whole blood or PBMC
was performed by FACS (Becton-Dickinson Immunocy-
tometry, San Jose, California, USA) by using one,
two or three of the following mAb’s conjugated with
FITC, PE, or peridin chlorophyll protein (PerCP): CD3,
CD4, CD8, HLA-DR, CD45RA, CD45RO (DAKO A/S,
Glostrup, Denmark), and CD28 (Becton-Dickinson
Immunocytmometry). Intracellular expression
of CTLA-4 with PE-conjugated mouse anti-human
CD152/CTLA-4 mAb (PharMingen, San Diego, Cali-
ifornia, USA) in CD4+ cells was performed with the
Ortho Permeafix reagent (Ortho Diagnostic Systems
Inc., Raritan, New Jersey, USA) according to the
manufacturer’s instructions. Cells incubated with FITC,-
PE-, or PerCP-conjugated mouse IgG1/IgG2a (DAKO)
served as isotype controls. A minimum of 10,000 cells
per sample was analyzed.

Cell extracts. PBMCs (2 × 10^6 cells) in 1 ml of RPMI
medium were stimulated with 1 μM Ca-ionophore
A23187 (Sigma) and 10 ng/ml PMA. At various time
intervals after stimulation, the cells were washed with
cold PBS and resuspended in 50 μl of cold 10 mM
HEPES buffer (pH 7.6) containing 10 mM KCl, 1 mM
DTT, 1 mM EDTA, 0.1 mM EGTA, 2 mM Na3VO4, 2
mM PMSF, 5 μg/ml leupeptin, 0.3 U/ml aprotinin, 5
μg/ml pepstatin, and 20 mM β-glycerophosphate. After
15 minutes of incubation on ice, NP-40 was added to the cells to a final concentration of 0.6%, and the cells were incubated on ice for an additional 5 minutes. The mixture was then centrifuged at 21,000 g at 4°C, and the supernatant was kept at −20°C until tested. When cell extracts were prepared for examination of phosphorylation of IκB, the cells were incubated for 90 minutes with 150 μM N-acetyl-Leu-Leu-norleucinal (ALLN) before their stimulation. All used reagents were obtained from Sigma.

Antibodies and immunoblotting. Aliquots of the cytosolic extracts (corresponding to -7×10^6 cells) were electrophoresed on SDS–10% polyacrylamide gels and transferred to nitrocellulose blotting membrane (BioTrace NT; Gelman Sciences, Ann Arbor, Michigan, USA). The membranes were incubated with the primary Ab for 1 hour at room temperature or overnight at 4°C and then blocked for at least 1 hour with 5% skim milk (Fluka BioChemika, Buchs, Switzerland). The membranes were then incubated for 1 hour at RT with anti-mouse or anti-rabbit HRP Ab and analyzed by using the chemiluminescent SuperSignal substrate according to the manufacturer’s instructions (Pierce Chemical Co., Rockford, Illinois, USA). The following primary and secondary antibodies were used in immunoblotting at the dilutions indicated: rabbit anti-IκBα (1:2,500; a kind gift from Y. Ben-Neriah, Hebrew University, Jerusalem, Israel); mAb anti-p42/44 mitogen-activated protein kinase extracellular signal-regulated (MAPK/ERK) and mAb anti-phosphorylated p42/44 MAPK/ERK Ab (1:10,000; both a generous gift from R. Seger, Weizmann Institute, Rehovot, Israel); anti-BCL2 Ab (1:500; DAKO A/S); 4G10 mAb antiphosphotyrosine Ab (1:1,000; Upstate Biotechnology Inc., Lake Placid, New York, USA); and anti-mouse IgG HRP conjugate (1:3,000) purchased from Promega Corp. (Madison, Wisconsin, USA); mAb anti-diphosphorylated p38 MAP kinase Ab (1:1,000) and HRP-conjugated anti-rabbit IgG (1:10,000) were all purchased from Sigma.

PPD skin test. DTH tests against PPD were carried out by intracutaneous administration, in the flexor surface of the forearm, of 5 tuberculin units (0.1 ml) of the standard strength tuberculin test (Mantoux, Tubersol; Connaught Laboratories Ltd., Toronto, Ontario, Canada). The PPD DTH test is carried out routinely in the Kaplan Medical Center.

Statistical analysis. Statistical analysis of the data was carried by using SigmaStat for Windows Statistical Software (version 2.0; Jandel Scientific, Chicago, Illinois, USA). Spearman Rank correlations, Mann-Whitney rank sum tests and McNemar’s χ² tests were used to analyze the data and compare the groups.

Results

Immune profile of the studied population. The immune profile of 111 new-Eth., 48 old-Eth., and 31 Isr. individuals (summarized in Table 1) revealed a clear dysregulation of several immune parameters in the new-Eth. group compared with the Isr. group. Such wide dysregulation is similar to that observed previously by us in other new-Eth. individuals (5–7). In contrast, the differences between the old-Eth. group and the Isr. group are not so remarkable (Table 1), demonstrating that the immune profiles of the Eth. individuals return gradually to normal after their immigration to Israel.

For further analysis, and for the sake of simplicity, we divided the examined individuals into three categories of activation, according to the proportion of HLA-DR⁺ cells within the CD3⁺ cell population and the proportion of CD28⁺ cells within the CD8⁺ cell population. Nonactivated (NA) individuals were defined as those having a normal percentage of HLA-DR⁺/CD3⁺ and CD28⁺/CD8⁺ cells (similar to that of the Isr. group, i.e., <9% HLA-DR⁺/CD3⁺ cells and >40% CD28⁺/CD8⁺). Highly activated (HA) individuals were defined as those having more than 9% HLA-DR⁺/CD3⁺ cells and less than 40% CD28⁺/CD8⁺, whereas partly activated (PA) individuals were defined as those having either HLA-DR⁺/CD3⁺ or CD28⁺/CD8⁺ subsets above or below the normal range, accordingly. All members of the Isr. group were NA, and members of the new-Eth. group were either HA or PA. About 35% of the old-Eth. group were NA, while the rest were still PA.

Correlation between activation, naive and memory cells, expression of CD28 costimulatory molecules, and the number of CD4⁺ cells. We have previously suggested that the decreased CD4 levels and significantly lower CD4/CD8 ratio in the new-Eth. group, compared with the Isr. group, is a consequence of immune activation (5–7). Correlation studies between different immune parameters of the examined Eth. population in the present study strongly supports this hypothesis. For example, individuals with higher percent of HLA-DR⁺/CD4⁺ cells had less CD4⁺ cells in the peripheral blood, lower CD4/CD8 ratio, lower percentage of CD28⁺/CD8⁺, naive (CD45RA⁺) cells, and higher percentage of memory (CD45RO⁺) cells (Figure 1). Similar correlations between HLA-DR expression on CD8⁺ and CD3⁺ cells and the various immunological markers examined were also found (data not shown).

Signal transduction studies. Physiological activation of T-lymphocytes requires costimulation through the T-cell receptor/CD3 complex and CD28, which can be mimicked by PMA in combination with Ca²⁺ ionophore (16–18). After such costimulation, a cascade of phosphorylations and dephosphorylations of intracellular protein occurs, which include the MAPK/ERK, p38 (19, 20), and IκBα proteins (13, 14). The IκBα, once phosphorylated, are degraded by cytoplasmic proteases, enabling the translocation of NF-κB into the nucleus (21, 22).

To study and characterize the effect of immune activation on T-cell signaling, we isolated PBMCs from 10 NA (Isr. and Eth.), 11 PA (Eth.), and 12 HA (Eth.) individuals and stimulated them with PMA and Ca²⁺ ionophore. At various time intervals after stimula-
Table 1

Immune profile of studied populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Isr.</th>
<th>new-Eth.</th>
<th>old-Eth.</th>
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<tbody>
<tr>
<td>CD4+ (cells/μl)</td>
<td>853 ± 44</td>
<td>702 ± 21.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>749 ± 41.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD8+ (cells/μl)</td>
<td>638 ± 34</td>
<td>757 ± 37.4</td>
<td>566 ± 28.4</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>1.38 ± 0.07</td>
<td>1.06 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.38 ± 0.06</td>
</tr>
<tr>
<td>CD45RA+/CD4+</td>
<td>35.2%</td>
<td>32.7 ± 40.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CD28+/CD8+</td>
<td>55.7%</td>
<td>56.3 ± 14.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>HLA-DR+/CD8+</td>
<td>5.9%</td>
<td>6.4 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>HLA-DR+/CD4+</td>
<td>3.0%</td>
<td>3.4 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CD45RA+/CD8+</td>
<td>65.8%</td>
<td>62.6 ± 14.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CD45RO+/CD8+</td>
<td>34.1%</td>
<td>31.5 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CD45RA*/CD8*</td>
<td>35.2 ± 2.2</td>
<td>24.2 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CD45RO*/CD4*</td>
<td>58.2 ± 1.54</td>
<td>68.3 ± 1.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CD45RO*/CD8*</td>
<td>34.1 ± 1.9</td>
<td>31.5 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>CD45RA*/CD8*</td>
<td>65.8 ± 2.46</td>
<td>61.5 ± 1.38</td>
<td></td>
</tr>
<tr>
<td>CD28*/CD8*</td>
<td>55.7 ± 2.8</td>
<td>40.2 ± 1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
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</table>

<sup>a</sup>P < 0.01; <sup>b</sup>P < 0.001; <sup>c</sup>P < 0.05.

The results are the mean ± SE obtained from 31, 111, and 48 Isr., new-Eth., and old-Eth. individuals, respectively. The significance of the difference between the Eth. groups and the Isr. group was determined by the Student’s t test. 
P < 0.01; <sup>b</sup>P < 0.001; <sup>c</sup>P < 0.05.

In five Isr. individuals examined (Figure 3b), indicating dephosphorylation by intracellular phosphatases. In contrast, no dephosphorylation of p38 kinase occurred in all five HA Eth. individuals examined (Figure 3b). In some experiments, as shown in a representative case in Figure 3c, only slight p38 phosphorylation occurred in the cell extracts from HA Eth. individuals; but in any case, no changes were noted after the cell costimulation by Ca<sup>2+</sup> ionophore and PMA in these samples. Hence, we did not note p38 phosphorylation or dephosphorylation in the case of the HA Eth. individuals.

Analyses of the phosphorylation of phosphotyrosine kinases revealed a similar pattern of hyporesponsiveness in ten HA and PA Eth. individuals examined, as opposed to ten NA Eth. and NA Isr. individuals examined. In the case of NA individuals, a significant decrease of phosphorylated kinases was noted 3 minutes after the cell stimulation; thereafter, phosphorylated kinases continued to decrease in a somewhat lower rate. No such decrease was noted in the cell extracts obtained from activated individuals, indicating that they did not respond to the stimuli (Figure 4).

β-Chemokine secretion of CD8<sup>+</sup> cells after stimulation by phytohemagglutinin and PMA. The above signal transduction studies indicated altered capacity of PBMC obtained from immune-activated individuals to respond to stimuli, such as costimulation with Ca<sup>2+</sup> ionophore and PMA. Measurement of β-chemokine secretion by CD8<sup>+</sup> T cells stimulated for 48 hours with PMA and phytohemagglutinin (PHA), revealed a significantly lower secretion of RANTES and MIP-1α from cells obtained from 23 HA and PA Eth. individuals than from cells obtained from 16 Isr. individuals (27.8 ± 11.9 ng/ml vs. 78.1 ± 46.7 ng/ml of RANTES secretion; P < 0.0001; and 95.5 ± 38.3 ng/ml vs. 137.7 ± 48.9 ng/ml of MIP-1α secretion; P < 0.02).

Increased intracellular pool of CTLA-4 in the Eth. group. CTLA-4 is a CD28 homologue that serves as a negative regulator of T-cell responses (23, 24). Binding of B7 to CTLA-4 inhibits the CD28-dependent IL-2 production and proliferation (23, 25). Furthermore, ligation of CTLA-4 receptor by B-7 selectively shuts off activation of downstream T-cell receptor/CD28 signaling events, such as activation of ERK and JNK kinases (26). Resting memory cells, but not naive cells, contain intracellular supplies of CTLA-4 that are continuously recycled between the cytoplasm and the cell surface, raising the cells threshold for costimulatory signals (24). As PBMCs obtained from immune-activated Eth. individuals had decreased capacity to respond to stimuli, we examined whether they have increased expression of CTLA-4. Indeed, a comparison between CTLA-4 expression in 13 PA and HA Eth. and 24 NA Isr. subjects revealed significantly higher expression of CTLA-4 in the Eth. than in the Isr. subjects (8.47 ± 1.97% vs. 5.29 ± 2.47% CTLA-4<sup>+</sup>CD4<sup>+</sup> cells, respectively; P = 0.03).
lived in areas endemic to TB, we examined the capacity of PBMCs obtained from activated and nonactivated Eth. individuals to proliferate after stimulation with PPD, a TB-specific antigen. As shown in Figure 5a, there is a significant, although weak, inverse correlation between the capacity of the cells to proliferate following PPD stimulation and the percentage of HLA-DR+/CD3+ cells. It should be noted that in all cases in which PBMCs were obtained from HA Eth. individuals, they proliferated poorly after PPD stimulation, and significantly less than in all other Eth. or PA individuals (P < 0.001 and P = 0.038, respectively, according to the Mann-Whitney rank sum test). Those that were able to respond significantly to the recall antigen were either NA or PA Eth. individuals.

As CTLA-4 was elevated in Eth. compared with Isr. subjects (see earlier discussion), and as CTLA-4 is a negative regulator of T-cell responses (23, 24) and upregulates the cell’s threshold for costimulatory signals (24), we examined whether blocking of CTLA-4 with Fab mAb would enhance the proliferative responses to PPD of PBMCs obtained from Eth. subjects. Indeed, as depicted in Figure 5b, PBMCs obtained from seven of eight Eth. subjects proliferated significantly more strongly to PPD in the presence of Fab mAb against CTLA-4.

DTH responses after intracutaneous PPD skin injections. DTH responses are part of the cell-mediated immunity. To study further the effect of immune activation on cell-mediated immunity, DTH responses to PPD intracutaneous injection (Mantoux), were examined in 39 HA or PA Eth. individuals, heavily infested with helminthic parasites, before and 6 months after receiving antihelminthic treatment (albendazole). In addition, the presence of parasites in their stool was examined at both time points. Importantly, the DTH-positive responses to PPD in those individuals in whom the helminths were eradicated increased significantly after deworming, as opposed to those in whom the helminths were not eradicated (P < 0.004, McNemar’s χ² test).

Discussion

Millions of individuals in the developing countries, especially in Africa, Southeast Asia, and South America, are infested with chronic infectious diseases, such as malaria, TB, protozoa and helminthic parasites, and HIV (1–3). The constant and lifelong confrontation of these hosts with such infectious burden results in a chronic immune activation and unbalanced immune state (5, 11, 27, 28). We have suggested that the capacity of such individuals to protect themselves and to cope with infections, as well as to mount protective immunity after vaccination, is highly impaired (5, 27).

The results of the present work strongly supports these suggestions by showing that chronic immune activation leads to hyporesponsiveness and anergy. This was manifested in highly immune-activated individuals by (a) defective or lacking of early transmembrane signaling (phosphorylation and/or dephosphorylation of tyrosine kinases), suggesting malfunction of both kinases and phosphatases; (b) lack of degradation of phosphorylated IkBα, indicating reduced or malfunction of a cellular protease(s); (c) lack or attenuated phosphorylation of downstream kinases, such as ERK and p38; (d) increased expression of CTLA-4, a downregulator protein; (e) decreased β-chemokine secretion of CD8+ cells after stimulation; (f) reduced capacity to proliferate after recall antigen stimulation; (g) restoration of proliferative responses after CTLA-4 blocking; and (h) decreased DTH responses to recall antigen before deworming. These attenuated responses were not a reflection of slower kinetics, but represented significantly diminished responses. Hyporesponsiveness did not occur in only one signal transduction pathway, but in all those that we examined, indicating that this phenomena is a more general cell deficiency.

In concordance with these results are the following observations made by others: defective T-cell signaling occurs in patients with primary intracranial tumors (29); patients with malignant glioblastomas exhibit T-cell anergy (30); anergic Th1 murine cells have decreased p38 and ERK/MAPK activities, even though the levels of these proteins remain unchanged (31–33); and murine anergic CD4+ T cells are defective in their ability to...
upregulate protein binding and transactivation of the NF-AT and AP-1 transcription factors (34, 35).

T-cell receptor stimulation in the absence of CD28-mediated costimulation not only results in little IL-2 production, but induces a long-lasting hyporesponsive state known as T-cell clonal anergy (36). We have already found in several cohorts of Ethiopian immigrants to Israel that CD28+ expression on CD8+ cells was significantly reduced (5–7, 37). Also in the present work, the new- and old-Eth. cohorts studied had 30% and 10% lower expression of CD28+CD8+ cells, respectively, than did the Isr. group. This lower expression of CD28+ on CD8+ cells was negatively correlated with HLA-DR expression in all T-cell subsets. Clearly, the reduced expression of this important costimulatory molecule in T cells in chronically immune-activated individuals is detrimental to their capacity to mount immune responses.

Worsening the situation is the finding that the new-Eth. individuals studied had significantly less CD4+ cells, less naive (CD45RA+) cells, more memory (CD45RO+) cells, and increased CTLA-4 expression on CD4+ cells than did the Isr. cohort. This is in accordance with our previous findings with other Eth. cohorts studied (5–7, 37). These differences were correlated with the activation of the individuals (as manifested by the percentage of T cells expressing the surface marker HLA-DR). Clearly, the diminished number of CD4+ cells and the lower percentage of naive cells in these individuals lessen their capacity to mount effective immune responses.

A Th2-like immune response with concomitant anergy and downregulation of Th1-associated immunity has been shown in mice infected with a helminthic parasite (38). To our knowledge, this is the first example showing in a human population that constant immune challenge results in a wide immune dysregulation, a predominant Th2 type of cytokine profile, and a clear impairment in the capacity of their cells to respond to stimuli. Taken together, our study strongly supports the idea that chronic immune activation leads to hyporesponsiveness and deficiency of the immune cells, and in some extreme cases to anergy, endangering not only the capacity of these individuals to cope with infection but with their capacity to mount an effective immune response after vaccination.

Several other observations also lend further support to this notion; for example (a) the very high incidence of TB in Africa despite the wide Bacillus Calmette-Guerin (BCG) vaccination carried out in this continent (39, 40); (b) the faster HIV disease progression in sub-Saharan Africa compared with the Western world (41, 42); (c) increased HIV plasma viral load, observed in helminth-infested regions in Africa (43), which was associated with Leishmaniasis and decreased after its treatment (44, 45); (d) human individuals, chronically infected with the filarial parasite *Onchocerca vulvurus*, have depressed cellular reactivity in vitro and deficient production of IL-2 in response to *O. vulvurus*–specific antigenic stimulation (46); and (e) humans infected with *Schistosoma mansoni* have impaired tetanus toxoid-specific immune responses (47).
It should be noted that the expression of CD28+ on CD8+ cells, the CD4/CD8 ratio, and the percentage of naive and memory cells, as well as the in vitro capacity to respond to stimuli, returns gradually to normal in Eth. individuals living for prolonged periods in Israel. Obviously, the change in environment, the better health care, nutrition, and so forth, and most importantly the eradication of the helminthic parasites, with which more than 80% of the new-Eth. immigrants were afflicted, contributed to this improvement in the immune system of these individuals.

It is believed that generation of HIV- or M. tuberculosis–specific cellular immunity by a vaccine, via a Th1 immune response, is a major prerequisite for any protective HIV or M. tuberculosis vaccine (48–50). The highest incidence of both M. tuberculosis and HIV infections are in Southeast Asia and Africa (2, 3). It is clear that the efficacy of HIV and TB candidate vaccines will have to be tested in human field trials that can only take place in Africa and Asia, in areas with a high incidence of HIV and M. tuberculosis infections. However, potentially good vaccines may fail in such clinical trial if examined in the immune scenario presently existing in the developing world. It is quite clear that the host immune background in developing countries is biased toward a Th2 profile and that most individuals are in a chronic immune-activation state, similar to that which we have seen in the Eth. group. Our findings that signal transduction in such individuals is impaired and that their immune cells can respond poorly to stimuli, such as to PPD, suggest that their capacity to elicit an immune response after vaccination will be heavily encumbered. The failure of BCG vaccination in Africa and Asia to confer protective immunity to TB clearly supports this assumption. It therefore becomes essential to take this major issue into consideration for any protective vaccine development. The simplest and most effective way to do so is by eradication of helminth infections before or during vaccination. Such eradication is important by itself and has clear advantages and benefits regardless of the issue of HIV and TB vaccination, such as the capacity to cope with infections, the capacity to learn, and so on. We suggest that deworming will have a huge impact on the capacity of individuals in the developing world to cope with disease, and more importantly, we believe that without eradication of helminths, HIV and TB vaccines, and probably others, may fail to confer protection in helminth endemic areas.
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