

High prevalence of HIV-specific immunity in seronegative Ethiopian immigrants in Israel

The notion that exposure to HIV does not necessarily lead to infection is not new. There is by now strong epidemiological evidence indicating that a large proportion of individuals at high risk for infection, never get infected and remain HIV-seronegative [1-3]. An attractive explanation for these observations is the presence of specific and protective cellular immunity to HIV in these HIV-seronegative individuals. We have previously described the presence of HIV-specific humoral immunity in seronegative wives of HIV-infected Ethiopian immigrants to Israel [4]. Our interest in characterizing further the nature of HIV specific immunity in this African population led us to perform the present study.

We studied 131 HIV-seronegative Ethiopian immigrants who arrived in Israel in 1991. They comprised 35 sexual partners of HIV-seropositive individuals, 36 children (aged 1-3 years) born to HIV-seropositive mothers, and 60 immigrants with no known exposure to HIV. A group of 36 HIV-seronegative Israeli non-Ethiopians presumed unexposed served as negative controls, and HIV-seropositive carriers served as positive controls.

Plasma samples collected from all participants were assayed for HIV-1 antibodies by enzyme-linked immunosorbent assay (ELISA) and confirmed by Western blot. Polyclonal B-cell activation test (PBAT) was performed as previously described [4,5]. Briefly, peripheral blood mononuclear cells (PBMC) at 2×10^6 ml in RPMI medium supplemented with 10% heat-inactivated fetal calf serum, glutamine and antibiotics were cultured for 6 days in the presence of pokeweed mitogen at a titre of 1/400 of the stock. On day 6, the culture supernatant fluid was tested for the presence of antibodies to HIV-1 by ELISA and samples that tested positive were then tested by Western blot. The results were considered positive if there were bands for at least two major HIV proteins. Specific T-cell immune response (STIR) was performed as previously described in detail by Clerici *et al.* [6]. Briefly, peripheral blood mononuclear cells (PBMC) were exposed to each of five HIV peptides from the envelope (*env*) region (provided by J. Berzofsky, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA) for 7 days in culture, in the presence of anti-Tac. The control wells had either nothing added (negative control) or tetanus (recall antigen) or phytohaemagglutinin (PHA) to check for the presence of proliferative response of the PBMC sample (positive controls). Samples that showed no response to PHA were excluded from the study. The supernatant fluid

samples were tested for interleukin (IL)-2 activity by bioassay as described [7]. STIR was defined as positive when IL-2 generation was at least twofold of unstimulated cultures, and in response to at least two out of five HIV peptides. Polymerase chain reaction (PCR) was performed using a previously published method [8]. The primers used were SK 38 (nucleotides 1331-1378, *gag*) and SK 39 (nucleotides 1638-1665, *gag*) and the probe used was SK 70. HIV culture was performed by cocultivation of donor's PBMC with PHA-stimulated normal donor PBMC as described by Gupta *et al.* [9] and culture was considered positive with a minimum fourfold increase in p24 antigen to at least 100 pg.

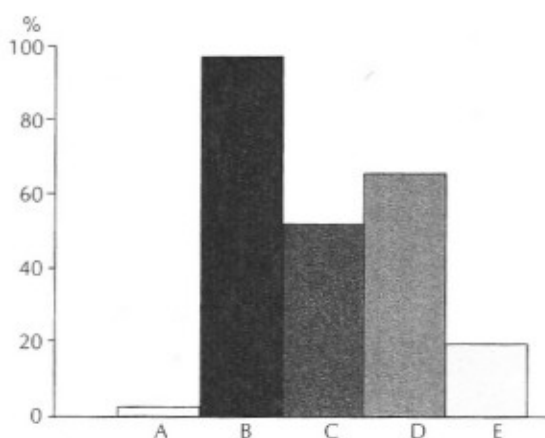


Fig. 1. HIV-specific immunity (polyclonal B-cell activation test-positive, specific T-cell immune response-positive, or both) in different studied groups. A, HIV-seronegative Israeli non-Ethiopians (negative control); B, HIV-seropositive Ethiopian immigrants (positive control); C, HIV-seronegative Ethiopian immigrants; D, sexual partners of HIV-seropositive Ethiopian immigrants; E, children born to HIV-seropositive Ethiopian immigrant mothers.

The results of these studies are shown in Fig. 1. HIV-specific immunity (STIR-positive, PBAT-positive, or both) was present in one out of 36 (2.8%) of seronegative non-Ethiopian Israeli controls, 37 out of 38 (97.4%) of HIV-seropositive Ethiopian immigrants, 23 out of 35 (65.7%) of seronegative sexual partners and seven out of 36 (19.4%) of seronegative children born to HIV-infected mothers. To our surprise, a very high proportion of positive HIV-specific immunity — 31 out of 60 (52%) — was detected among HIV-seronegative healthy Ethiopian immigrants (Fig. 1). In this group 40, 11.7 and 3.3% had STIR, PBAT, or both, respectively.

In order to better characterize the nature of this response, we focused subsequent studies on a smaller group of 21 individuals who were previously found positive for either PBAT, STIR, or both assays. Upon entering the study in early 1993, 71.5% were STIR-positive, 38% were PBAT-positive, and 9.5% were both STIR and PBAT-positive. One year later, while all participants remained HIV-seronegative, the study was repeated. This time a clear increase in the proportion of positive assays was evident with 95, 81 and 76% showing a positive STIR, PBAT, or both, respectively. Repeated PCR studies on samples obtained from all 21 participants were negative except in one and five samples in 1993 and 1994, respectively. All efforts to culture or rescue virus from PBMC samples obtained from these individuals, performed in Israel and abroad, were negative.

The results of this study show that in a large proportion of HIV-exposed yet seronegative Ethiopian immigrants significant cell-mediated HIV-specific immunity is demonstrable. Furthermore, such immunity was found in a surprisingly high proportion (>50%) of seronegative presumed unexposed healthy Ethiopian immigrants. On repeated studies of 21 such persistently HIV-seronegative, HIV-specific immunity-positive individuals, a progressive increase in that immunity over the course of 1 year of follow-up was found. Such specific immunity was not accompanied by seroconversion and could not be accounted by presence of HIV infection, although judging by the results of the five positive PCR determinations in this group the possibility that a small number of viral copies is present in all of the 21 individuals cannot be completely excluded.

The interpretation of these results is not entirely clear. The number of different HIV antigens to which a response was found is not small, since in the positive PBAT assay there was reactivity to two or more different bands of the HIV Western blot assay, and in the STIR assay there was a positive reaction to at least two additional different peptides. Thus, together they provide compelling evidence for the presence of genuine HIV-specific immunity in these individuals, probably exposed to HIV at some point. Alternatively, these reactions could possibly represent some cross antigenicity between HIV and other pathogens [10] and thus present a form of natural immunity in these individuals that could also be protective.

Is there something unique in the setting or background of the Ethiopian population that may account for these findings? We favour the idea that the high load of endemic infections and primarily the helminth infections present in Ethiopia may be responsible [11]. This would account for a different mode of handling of HIV, that following exposure to HIV, and due to the continuous activation of macrophages [12] antigen presence may be prolonged. On the other hand, it

could provide the background for cross-reaction with several pathogens. Studies to further characterize these interesting observations, especially by generation of cytotoxic T lymphocytes to HIV antigens, are now in progress.

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