Journal of Clinical Immunology, Vol. 24, No. 6, November 2004 (© 2004)

Elevation of Immune Activation in Kenyan Women is Associated with Alterations in Immune Function: Implications for Vaccine Development

SANDRA A. KOESTERS,[1] LUCY MATU,[2] PETER KIAMA,[2] OMU ANZALA,[2] JOANNE EMBREE,[1] FRANCIS A. PLUMMER,[1] JOSHUA KIMANI,[2] and KEITH R. FOWKE[1,3]

Accepted: July 7, 2004

The infectious burden leading to immune activation can vary between different populations and lead to various immune dysfunctions. We compared the effect of immune activation on apoptosis and T cell function in HIV uninfected individuals from Nairobi, Kenya ($n = 34$), and Winnipeg, Canada ($n = 10$). Women from Nairobi had a significantly greater number of CD8+ T cells expressing the activation markers CD38 and HLA DR. Kenyan women also had significantly higher levels of CTLA-4+ CD4 and CD8+ T cells, and reduced levels of CD28+ CD8+ cells. Levels of CD95+ CD4+ T cells were higher in Kenyan women and, correspondingly, showed higher levels of spontaneous apoptosis. Kenyan women also demonstrated hyper-responsiveness to T cell activation as assessed by interferon gamma production. This study demonstrates that in a population of Kenyan women with high levels of T cell activation, there were also elevated levels of T cell apoptotic death and hyper-responsiveness. These differences may influence the efficacy of immune responses to pathogens and must be considered when testing candidate vaccines.

KEY WORDS: Immune activation; apoptosis; population differences; T cell homeostasis; intracellular cytokine staining; flow cytometry; hyper-responsiveness.

INTRODUCTION

For the immune system to respond to antigenic challenge it must be able to become rapidly activated and then, just as quickly, return to a resting state. Immune activation is a collection of multiple specific immune responses which leads to increased expression of leukocyte activation markers such as HLA DR and CD38, production of pro-inflammatory cytokines, a rise in T-cell proliferation, apoptotic cell death, and the production, regulation, and maintenance of immunological memory (1). Although successful immune activation is essential to an effective immune response against pathogens, chronic immune activation is not beneficial. Chronic activation can lead to severe immune dysfunctions such as anergy, and enhanced activation-induced cell death (AICD) (2). There are a number of situations when levels of immune activation remain elevated. These include chronic viral infection such as HIV, rheumatic and autoimmune diseases and cancer (1). Elevated levels of immune activation have also been observed in aged population and in populations from developing nations (3–9).

One such study compared Ugandans and Italians and indicated that markers of immune activation are increased in Ugandan HIV negative individuals (6, 10). Another study showed newly immigrated HIV negative Ethiopians to Israel had elevated levels of HLA DR on CD4+ and CD8+ T lymphocytes and CD38 on CD8+ T lymphocytes in comparison to resident Israelis (11). A similar study comparing HIV negative Ethiopian and Dutch populations found the Ethiopians to have elevated levels of immune activation (9). Findings from these studies lead to the suggestion that immune activation is environmentally driven.

The majority (>70%) of those infected with HIV reside in Sub-Saharan Africa. It is clear that a preventative vaccine is urgently needed. Many vaccines, developed in western nations, have already gone into clinical trials in those countries. The problem, however, is that the effectiveness of vaccines can vary substantially between different populations. In one example, the BCG vaccine was found to be 80% effective in a group of young men in the

[1]Department of Medical Microbiology, University of Manitoba, William Avenue, Winnipeg, MB., Canada.
[2]Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya.
[3]To whom correspondence should be addressed at Department of Medical Microbiology, University of Manitoba, 539–730 William Avenue, Winnipeg, MB., Canada, R3E 0W3; e-mail: fowkekr@cc.umanitoba.ca

United Kingdom but showed little to no efficacy in an age and sex matched group from Malawi (12). The ability of an individual to mount a rapid and vigorous response to a previously encountered antigen is the trademark of the immune system and the basis of vaccinations. However, the state of the immune system at the time of vaccination is key in determining the success of the immune response, yet it remains understudied (13).

As the infectious burden varies between different populations, we chose to investigate the effects of immune activation on HIV negative individuals from two distinct geographic locations; Nairobi, Kenya, and Winnipeg, Canada. In this study we not only look at the immune activation profiles but also T cell functional outcomes. Because of the known effects of HIV infection on immune activation (14), this study focused on HIV uninfected individuals, thereby removing HIV as a confounding variable. We measured surface markers of activation (HLA DR, CD38) and co-stimulation (CTLA-4, CD28), as well as expression of a death receptor (CD95). Functional measures included levels of spontaneous apoptosis and interferon gamma (IFN-γ) in response to Stapylococcus enterotoxin B (SEB) stimulation. There are few studies in the literature that simultaneously measure immune activation and assess functional characteristics, particularly in this type of population comparison. Our results indicate that Kenyan women had significantly increased immune activation and, more importantly, had altered immune functions when compared to the Canadian group. This may have impacts on the efficacy of vaccine strategies and must be considered when testing vaccine candidates.

METHODS

Study Subjects and Samples

Study subjects were female donors from the Pumwani Commercial Sex Workers (CSW) Cohort ($n = 23$), and the Pumwani Mother Child Health antenatal cohort (non sex worker cohort—NSW) ($n = 11$), both located in Nairobi, Kenya. Sex and age-matched healthy lab volunteers from Winnipeg, Canada ($n = 10$) were also utilized. All samples from HIV negative individuals were assessed by HIV serology. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood by density gradient centrifugation using ficol–hypaque (Bio-Lynx, Gibco) and washed twice in RPMI (Sigma) containing 10% FBS (Gibco).

Immunophenotyping

PBMC isolated from whole blood were blocked with 1 ug/mL of human gamma globulin for 10 min. Cells were then incubated with a panel of fluorochrome-conjugated antibodies. These included anti-CD4 (PerCP), anti-CD8 (APC), anti-CD152 (CTLA-4) (FITC), anti-CD28 (PE), anti-HLA DR (FITC), anti-CD38 (PE). All antibodies for this study were purchased from BD Pharmingen (San Diego, CA). After 30 min at 4^0 in the dark the cells were washed in PBS-2% FCS and resuspended in 1% paraformaldehyde (Sigma). The samples were analyzed using a FACSCalibur flow cytometer (Becton Dickenson).

Cell Death Assays

Spontaneous apoptosis was measured using freshly isolated PBMC. Cells were incubated with anti-CD4 or anti-CD8 (PE) and anti-CD95 (APC). They were then washed with PBS-2% FCS and resuspended in annexin binding buffer (10 mM Hepes, 140 mM NaCl, 2.5 M CaCl2, pH 7.4). Cells were then incubated with annexinV (FITC) for 15 min in the dark before analysis.

Intracellular Cytokine Assays

Freshly isolated PBMC were stimulated with SEB (5 ug/mL, Sigma) for 2 h before addition of 10 ug/mL Golgi Plug (BD Pharmingen). The cells were cultured overnight in RPMI (Sigma) containing 10% FCS (Gibco). Cells were then stained for surface markers using anti-CD4 (PerCP), anti-CD8 (APC), and anti-IFN-γ (FITC) using the BD Fix-Perm kits and protocol before being analyzed.

Statistical Analysis

Mean values (percent cells expressing a particular phenotype) were compared between groups using the nonparametric Mann-Whitney test. All the p values less than 0.05 were considered significant.

RESULTS

Assessment of T Cell Activation Levels

HLA-DR and CD38 expression was measured as an indication of T cell activation (Fig. 1). The proportion of cells expressing HLA-DR was similar in the CD4+ T cell subset of both populations, however, in CD8+ T cell subset it was significantly higher in the Kenyan group ($p = 0.041$). A similar pattern was seen with CD38, an immune activation marker specific for CD8+ T cells, in that the Kenyan women had more cells expressing CD38 in the CD8+ T cell subset ($p < 0.001$), while in the CD4+ subset there were no significant differences between groups.

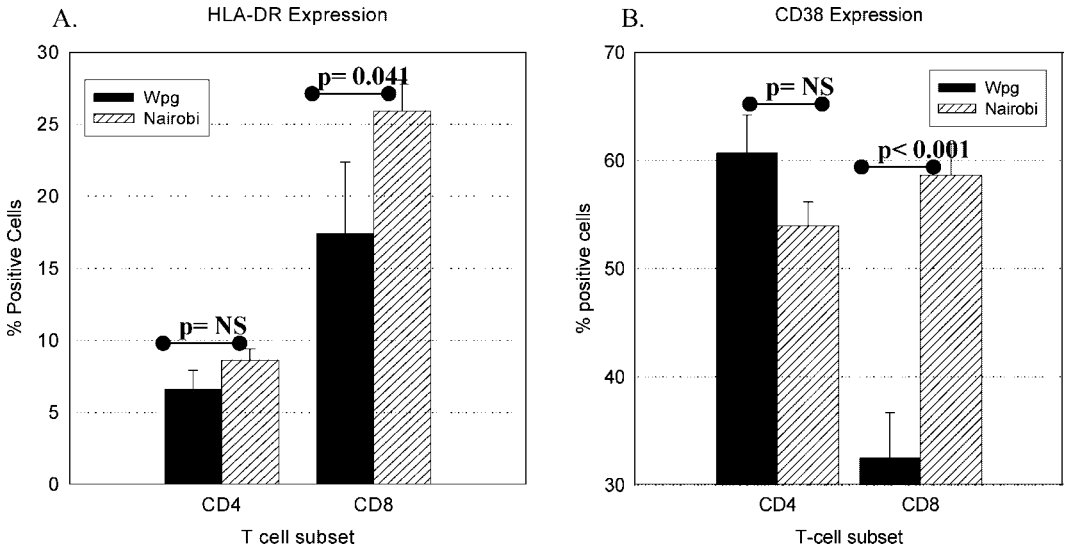

Fig. 1. Expression of markers of immune activation. PBMC were isolated and stained immediately. (A). Percentage of CD8+ T cells expressing HLA DR on cells (CD4: $p = 0.121$, CD8: $p = 0.041$). (B). Expression of CD38 on the CD8 T-cells ($p < 0.001$). Bars represent the mean expression and error bars are standard error of the mean. The black bars is the Winnipeg group ($n = 10$) and the hatched bar is the Nairobi women ($n = 34$).

Evaluation of Co-Stimulatory Molecule Expression

Expression of co-stimulatory molecules CD28 and CTLA-4 (CD152) were measured on the surface of CD4+ and CD8+ T cells (Fig. 2). In the CD4+ T cell subset, the two groups had a similar proportion of cells expressing CD28, however, the Kenyan women had more cells that expressed CTLA-4 ($p = 0.001$). In the CD8+ T cell subset, the Kenyans expressed lower levels of CD28 and had higher expression of CTLA-4 compared to the Winnipeg group ($p = 0.006$; $p < 0.001$, respectively).

Apoptosis and Death Receptor Expression

Expression of the death receptor CD95 was measured on the CD4+ and CD8+ T cell subsets. A higher

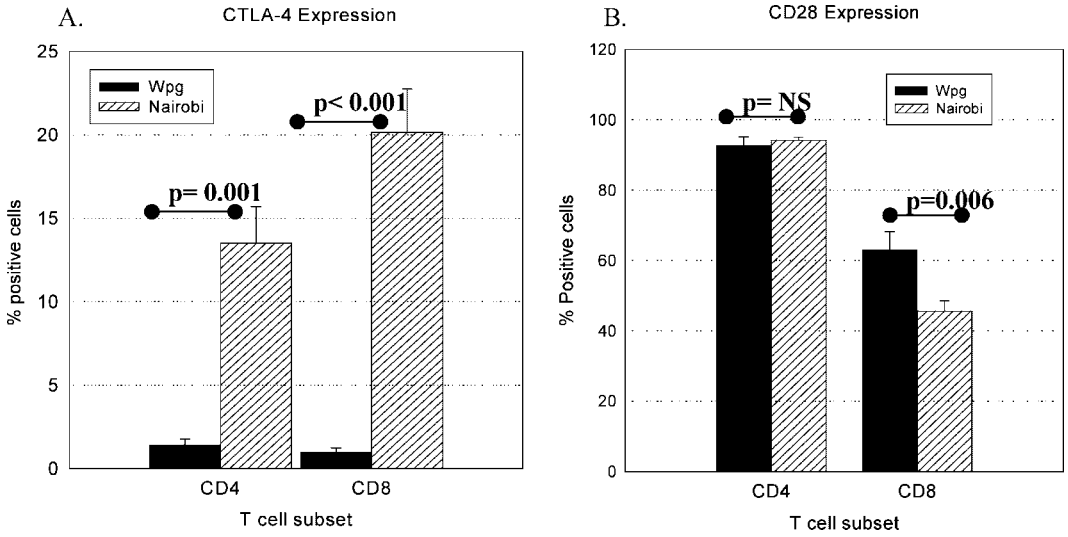

Fig. 2. Expression of co-signalling molecules. (A). Expression of CTLA-4 between the two study groups (CD4: $p = 0.001$, CD8: $p < 0.001$). (B). Expression of CD28 on T cells (CD8: $p = 0.006$). Bars represent the mean expression and error bars are standard error of the mean. The black bars is the Winnipeg group ($n = 10$) and the hatched bar is the Nairobi women ($n = 34$).

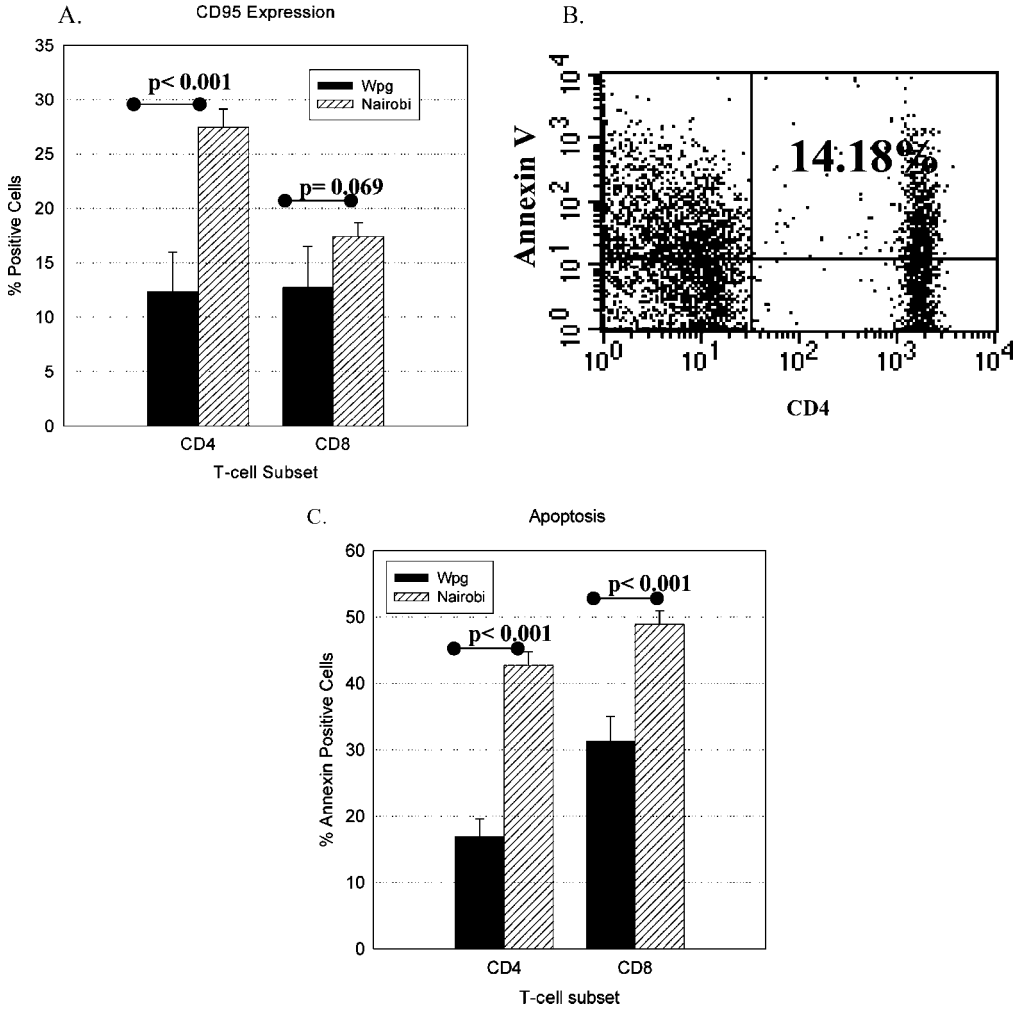

Fig. 3. Expression of death receptor and level of apoptosis. (A). Expression of CD95 on T cell subsets (CD4: $p < 0.001$, CD8: $p = 0.069$). (B). A representative dot plot of ex-vivo cell death assessed by CD4+ annexin co-staining. The percentage of CD4+ cells that were undergoing cell death is indicated in the top right quadrant. (C). Comparison of cells staining with Annexin-V (CD4: $p < 0.001$, CD8: $p < 0.001$). Bars represent the mean expression and error bars are standard error of the mean. The black bars is the Winnipeg group ($n = 10$) and the hatched bar is the Nairobi women ($n = 34$).

proportion of cells expressed CD95 in the CD4+ T cell subset of women from Nairobi when compared to women from Winnipeg ($p < 0.0001$) (Fig. 3). For a functional assessment of apoptosis, cells were measured for the surface expression of phosphotidylserine by the binding of fluorescently labeled annexin V. Both the CD4+ and CD8+ T cell subsets of the Kenyan group showed significantly more cells binding annexin V ($p < 0.001$ for both subsets).

Assessment of T Cell Function

To determine whether elevated immune activation markers had any effect on the ability of T cells to become stimulated through the T cell receptor, IFN-γ production in response to superantigen (SEB) stimulation was measured by intracellular cytokine staining of CD4+ and CD8+ T cells (Fig. 4). Following stimulation the mean number of IFN-γ producing cells was significantly increased in the Nairobi group for both T cell subsets (CD4+ $p = 0.026$, CD8+ $p = 0.006$).

Comparison Within the Kenyan Group

In addition to the Kenyan versus North American comparisons, we also contrasted HIV negative individuals from the CSW cohort to the NSW cohort for all of our assayed parameters. We found no significant differences

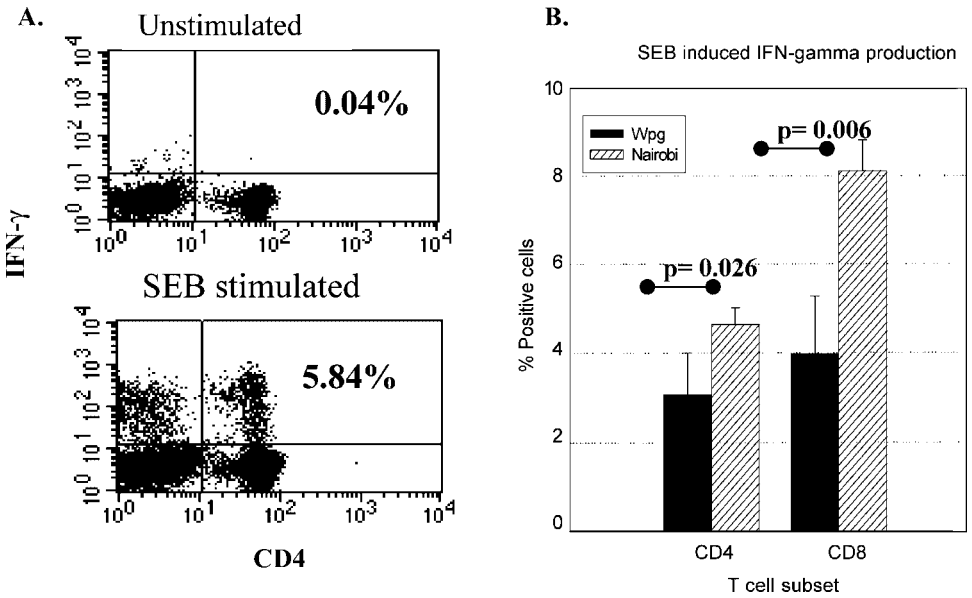

Fig. 4. Assessing T cell function. (A). Measures of T cell function by intracellular IFN-γ production. Cells were left unstimulated (top) or stimulated with SEB (bottom) and then stained for CD4 (shown) or CD8 and intracellular IFN-γ. The percentage of CD4/IFN-γ positive cells is indicated in the top right quadrant. (B). Hyper-responsiveness of the Pumwani group. Increased intracellular IFN-γ production in response to SEB superantigen is seen in both T cell subsets of the Kenyan women (CD4: $p = 0.026$, CD8: $p = 0.006$). Bars represent the mean expression and error bars are standard error of the mean. The black bars is the Winnipeg group ($n = 10$) and the hatched bar is the Nairobi women ($n = 34$).

in relative levels of activation (as assessed by HLA DR and CD38 expression), apoptosis, or IFN-γ production between these groups. Expression of CD28 was significantly altered between Kenyan groups (Fig. 5). Individuals from the CSW cohort had significantly higher expression of CD28 on both T cell subsets (CD4+ $p = 0.026$, CD8+ $p = 0.003$).

DISCUSSION

We have shown that HIV uninfected women from Nairobi have significantly more immune activation, apoptosis, and hyper-responsiveness when compared to a similar group in Winnipeg. This study found that women from Nairobi had more CD8+ T cells that expressed CD38+ and HLADR+ and fewer that expressed CD28+. On both CD4+ and CD8+ T cells, women from Nairobi had more cells expressing the death receptor CD95 and, correspondingly, had more apoptosis. We also saw significantly increased IFN-γ production in response to SEB stimulation in Kenyan women. Since the HIV negative Kenyan women represent a diverse group comprising both commercial sex workers and non sex workers, we compared immune activation levels within these populations and found CD28 expression to be the only parameter that was significantly different between the groups. Despite this difference, when the North American group was compared

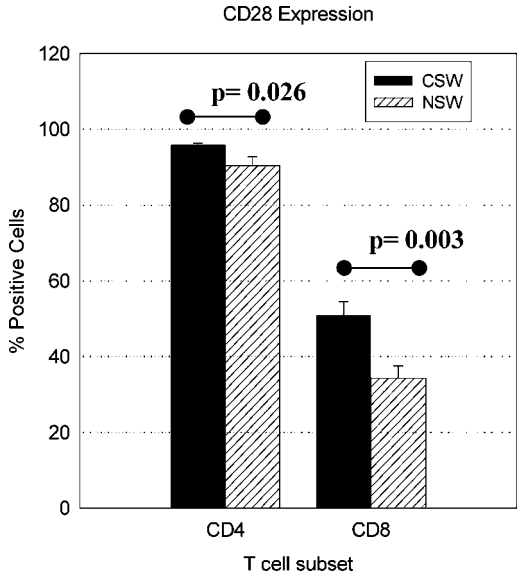

Fig. 5. Intra-Kenyan comparison. Expression of CD28 on T cells (CD4: $p = 0.026$, CD8: $p = 0.003$) Bars represent the mean expression and error bars are standard error of the mean. The black bars represents the commercial sexworker (CSW) group ($n = 11$) and the hatched bar represents women in the non-sexworker (NSW) group ($n = 23$).

to each Kenyan group independently for CD28 expression, the same findings were observed (no difference in CD28 expression on CD4+ T cells and higher CD28 expression on CD8+ T cells from the Winnipeg subjects). In fact, for all parameters studied when the North American group was compared to each Kenyan group independently, all reported significant results remained intact. As expected, when the two Kenyan groups were combined and compared with the North American group the significance increased due the larger data set. Although there may be subtle difference between Kenyan groups, relative to the North American group there are clearly high levels of immune activation in all Kenyan subjects studied. These high levels of immune activation in the Kenyan population may lead to immune dysfunctions such as anergy, impaired signaling, cytokine dysregulations, and activation induced cell death (8, 14–16).

The products of immune activation may also affect the migration, viability, and functional characteristics of resting lymphocytes (1, 17). It is possible that the increased immune activation seen in the Kenyan women may influence the course of HIV disease progression; should they become infected? It also could hinder the immune responses to other challenges including vaccines designed to confer protection to agents such as HIV, which is of particular importance to this population.

There are a number of reasons that may account for the increased immune activation seen in Kenyan women. Individuals living in developing countries are exposed to a different spectrum of pathogens including bacterial, viral and helimenthic infections. In areas where there is limited access to health care these infections may be long lasting, causing chronic immune activation. Other contributing factors may include diet, access to clean water, economic status, and genetics. Previous studies have shown elevated levels of immune activation in African populations (6–9, 18). Our study extends these observations of elevated immune activation in individuals from developing nations and correlates it with effects on cell death and immune function.

Immune activation is a necessary and required part of a successful immune response. From the time when antigen presenting cells (APC) first encounter antigen-specific T-lymphocytes of the matching specificity, there is a two way communication that occurs between the cells that results in phenotypic changes in both cells. The activation of the lymphocytes occurs through primary stimulation of the T cell receptor by the pathogen peptide associated with the major histocompatibility complex (MHC). Co-stimulation is achieved through co-signaling molecules such as CD28 and CTLA-4 on the T lymphocyte interacting with their ligands CD80 and CD86 on the APC. The regulation and expression kinetics of these molecules is critical to effecting proper immune regulation. CTLA-4, which is a negative regulator of the immune response, is not expressed on resting T cells but is up-regulated following activation of the cell (19). Our result show in both the CD4+ and CD8+ T cell subsets, CTLA-4 is significantly increased in the Kenyan women (Fig. 2). The over-expression of CTLA-4 in the Kenyan group is highly indicative of an activated T cell population that is expressing a powerful negative regulator of T cell activation that could affect antigen-specific responses such as those needed to induce vaccine responses.

Immune activation of naïve T cells stimulates them to enter the memory T cell pools and lose the expression of CD28 (17, 20). The decreased expression of CD28 seen in the CD8 T cell subset of Kenyan women also suggests that more cells have been activated and matured, no longer requiring co-stimulation. In fact, other studies have shown a low level of CD4+ CD28+ T cells is predictive of progression and mortality in HIV infection (21). Persistent stimulation of naïve cells through chronic immune activation could lead to accelerated depletion of the naïve CD4 and CD8 T cell pools (17, 22) via apoptosis or differentiation towards a memory phenotype (1). Some have called this a premature aging of the immune system and it is commonly seen among elderly individuals and those with HIV disease (3).

In both HIV and aging there is a decrease in IL-2 production, increased IFN-γ, and increased susceptibility to AICD (23). This is likely reflective of a shift in the T cell repertoire to an increasingly differentiated antigen-experienced cell. The enrichment of terminally differentiated T cells seen during ageing is thought to be a consequence of immune activation over time leading to immunosenescence (3). As in our Kenyan group, elderly individuals show increased HLA-DR expression, reduced expression of the co-stimulatory molecule CD28, and fewer naïve T cells (5, 24). CTLA-4 is also up-regulated during aging and HIV disease, and is correlated to immune activation (19, 25). It is known that in elderly individuals there is a reduced capacity to mount effective immune responses, particularly to vaccines (26, 27). We hypothesize that in populations showing increased immune activation and immune dysregulation, there will also be a diminished capacity to mount effective immune responses. This is particularly important when considering potential HIV vaccine candidates and may play a factor in accelerating HIV disease progression, as previous groups have reported (4).

This study looked at some functional aspects of the immune system including levels of apoptosis and IFN-γ response to SEB stimulation. We found Kenyan women showed increased levels of IFN-γ production when

compared to the Canadian women. This increased IFN-γ production is supported by the reduced levels of CD28 seen in the Kenyan women. It is possible that these cells represent the effector pool, which is responsible for immediate production of IFN-γ. The homeostasis of this memory pool is driven by the state of immune activation, where higher levels of activation drive cells from the naïve pool into the memory pool (1). The increased IFN-γ seen in the Kenyan women may be a measure of increased effector pools as a result of increased immune activation.

We also found that in the immune activated Kenyan women there was an increase in expression of the death receptor CD95, and correspondingly more apoptosis was seen, particularly in the CD4 T cell compartment. Activation-induced cell death (AICD) is a form of apoptosis that occurs as a consequence of repeated stimulation through the T-cell receptor (TCR) of T-cells and is a normal regulatory mechanism to limit an activated immune response. In the absence of HIV infection there is only one previous study that directly links the observation of elevated levels of immune activation with activation induced cell death (28). Here we show that in the immune activated Kenyans, there is also more T cell apoptosis. How this antigen activated cell death affects the degree to which an antigen-specific response may be generated is not known.

All of the phenotypic and functional immune differences we have observed between Kenyan and Canadian women may significantly influence the ability to mount a successful immune responses. Differences in vaccine efficacy between populations has been observed (12). It is highly likely that differences in immune activation leading to functional dysregulations such as excessive AICD, anergy, and altering of T cell memory homeostasis will have profound impact on the success of potential HIV vaccines.

In conclusion, we present both phenotypic and functional data which indicate that immune activation in a population from a developing nation has dramatic impacts on the responsiveness of the immune system. We have shown that relative to an age and sex matched group from Winnipeg, women from Nairobi have elevated levels of markers of immune activation, increased effector responses, and elevated levels of apoptosis when compared to women from Winnipeg, Canada. Given that not much is known about the role of elevated immune activation in interfering with vaccine efficacy, the immune status of individuals living in developing countries must be seriously considered during the development and testing of potential vaccines. Vaccine efficacy is likely to be population specific and vaccines which are successful in North American populations may not be effective in African populations.

ACKNOWLEDGMENT

Special thanks to the study participants and staff at the Nairobi clinics. Funding was provided by the Manitoba Health Research Council (MHRC) and the Canadian Institute for Health Research (CIHR) (HOP-43798). SAK is supported by a scholarship from the Canadian Institutes for Health Research and the International Centre for Infectious Diseases Training Program. FAP is a Canada Research Chair.

REFERENCES

1. Hazenberg MD, Hamann D, Schuitemaker H, Miedema F: T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock. Nat Immunol 1: 285–289, 2000
2. Bentwich Z, Kalinkovich A, Weisman Z, Grossman Z: Immune activation in the context of HIV infection. Clin Exp Immunol 111: 1–2, 1998
3. Appay V, Rowland-Jones SL: Premature ageing of the immune system: The cause of AIDS? Trends Immunol 23: 580–585, 2002
4. Kalayjian RC, Landay A, Pollard RB, Taub DD, Gross BH, Francis IR, Sevin A, Pu M, Spritzler J, Chernoff M, Namkung A, Fox L, Martinez A, Waterman K, Fiscus SA, Sha B, Johnson D, Slater S, Rousseau F, Lederman MM: Age-related immune dysfunction in health and in human immunodeficiency virus (HIV) disease: Association of age and HIV infection with naive CD8+ cell depletion, reduced expression of CD28 on CD8+ cells, and reduced thymic volumes. J Infect Dis 187: 1924–1933, 2003
5. Tarazona R, DelaRosa O, Alonso C, Ostos B, Espejo J, Pena J, Solana R: Increased expression of NK cell markers on T lymphocytes in aging and chronic activation of the immune system reflects the accumulation of effector/senescent T cells. Mech Ageing Dev 121: 77–88, 2000
6. Rizzardini G, Trabattoni D, Saresella M, Piconi S, Lukwiya M, Declich S, Fabiani M, Ferrante P, Clerici M: Immune activation in HIV-infected African individuals. Italian-Ugandan AIDS cooperation program. Aids 12: 2387–2396, 1998
7. Clerici M, Butto S, Lukwiya M, Saresella M, Declich S, Trabattoni D, Pastori C, Piconi S, Fracasso C, Fabiani M, Ferrante P, Rizzardini G, Lopalco L: Immune activation in africa is environmentally-driven and is associated with upregulation of CCR5. Italian-Ugandan AIDS Project. Aids 14: 2083–2092, 2000
8. Lukwiya M, Rizzardini G, Trabattoni D, Piconi S, Saresella M, Declich S, Fossati S, Clerici M: Evaluation of immune activation in HIV-infected and uninfected African individuals by single-cell analysis of cytokine production. J Acquir Immune Defic Syndr 28: 429–436, 2001
9. Messele T, Abdulkadir M, Fontanet AL, Petros B, Hamann D, Koot M, Roos MT, Schellekens PT, Miedema F and Rinke de Wit TF: Reduced naive and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. Clin Exp Immunol 115: 443–450, 1999
10. Rizzardini G, Piconi S, Ruzzante S, Fusi ML, Lukwiya M, Declich S, Tamburini M, Villa ML, Fabiani M, Milazzo F, Clerici M: Immunological activation markers in the serum of African and European HIV-seropositive and seronegative individuals. Aids 10: 1535–1542, 1996

11. Kalinkovich A, Borkow G, Weisman Z, Tsimanis A, Stein M, Bentwich Z: Increased CCR5 and CXCR4 expression in Ethiopians living in Israel: Environmental and constitutive factors. Clin Immunol 100: 107–117, 2001
12. Black GF, Weir RE, Floyd S, Bliss L, Warndorff DK, Crampin AC, Ngwira B, Sichali L, Nazareth B, Blackwell JM, Branson K, Chaguluka SD, Donovan L, Jarman E, King E, Fine PE, Dockrell HM: BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: Two randomised controlled studies. Lancet 359: 1393–1401, 2002
13. Borkow G, Bentwich Z: Host background immunity and human immunodeficiency virus protective vaccines, a major consideration for vaccine efficacy in Africa and in developing countries. Clin Diagn Lab Immunol 9: 505–507, 2002
14. Alimonti JB, Ball TB, Fowke KR: Mechanisms of CD4+ T lymphocyte cell death in human immunodeficiency virus infection and AIDS. J Gen Virol 84: 1649–1661, 2003
15. Leng Q, Borkow G, Bentwich Z: Attenuated signaling associated with immune activation in HIV-1-infected individuals. Biochem Biophys Res Commun 298: 464–467, 2002
16. Borkow G, Leng Q, Weisman Z, Stein M, Galai N, Kalinkovich A, Bentwich Z: Chronic immune activation associated with intestinal helminth infections results in impaired signal transduction and anergy. J Clin Invest 106: 1053–1060, 2000
17. Grossman Z, Meier-Schellersheim M, Sousa AE, Victorino RM and Paul WE: CD4+ T-cell depletion in HIV infection: Are we closer to understanding the cause? Nat Med 8: 319–323, 2002
18. Bentwich Z, Kalinkovich A, Weisman Z: Immune activation is a dominant factor in the pathogenesis of African AIDS. Immunol Today 16: 187–191, 1995
19. Leng Q, Bentwich Z, Magen E, Kalinkovich A, Borkow G: CTLA-4 upregulation during HIV infection: Association with anergy and possible target for therapeutic intervention. Aids 16: 519–529, 2002
20. Douek DC, Picker LJ, Koup RA: T cell dynamics in HIV-1 infection. Annu Rev Immunol 21: 265–304, 2003
21. Ostrowski SR, Gerstoft J, Pedersen BK, Ullum H: A low level of CD4+ CD28+ T cells is an independent predictor of high mortality in human immunodeficiency virus type 1-infected patients. J Infect Dis 187: 1726–1734, 2003
22. Hazenberg MD, Otto SA, Hamann D, Roos MT, Schuitemaker H, de Boer RJ, Miedema F: Depletion of naive CD4 T cells by CXCR4-using HIV-1 variants occurs mainly through increased T-cell death and activation. Aids 17: 1419–1424, 2003
23. Bandres E, Merino J, Vazquez B, Inoges S, Moreno C, Subira ML, Sanchez-Ibarrola A: The increase of IFN-γ production through aging correlates with the expanded CD8(+high)CD28(−)CD57(+) subpopulation. Clin Immunol 96: 230–235, 2000
24. Ginaldi L, De Martinis M, Modesti M, Loreto F, Corsi MP, Quaglino D: Immunophenotypical changes of T lymphocytes in the elderly. Gerontology 46: 242–248, 2000
25. Leng Q, Bentwich Z, Borkow G: CTLA-4 upregulation during aging. Mech Ageing Dev 123: 1419–1421, 2002
26. Hainz U, Aigner K, Asch E, Berger P, Bohmer F, Feldkircher B, Horwath B, Jenewein B, Kassal H, Kistner O, Mack H, Pfeiffer KP, Pils K, Plank J, Renner D, Saurwein-Teissl M, Schwanzer E, Trieb K, Grubeck-Loebenstein B: Vaccine protection in the elderly: Are Austrian seniors adequately protected by vaccinations? Wien Klin Wochenschr 114: 187–193, 2002
27. Stepanova L, Naykhin A, Kolmskog C, Jonson G, Barantceva I, Bichurina M, Kubar O, Linde A: The humoral response to live and inactivated influenza vaccines administered alone and in combination to young adults and elderly. J Clin Virol 24: 193–201, 2002
28. Kalinkovich A, Weisman Z, Greenberg Z, Nahmias J, Eitan S, Stein M, Bentwich Z: Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. Clin Exp Immunol 114: 414–421, 1998