Natural T, γδ, and NK Cells in Mycobacterial, *Salmonella,* and Human Immunodeficiency Virus Infections

Janine Jason,[1] Ian Buchanan,[1] Lennox K. Archibald,[3] Okey C. Nwanyanwu,[4] Michael Bell,[3] Timothy A. Green,[2] Angelia Eick,[1] Alison Han,[1] Dustin Razsi,[1] Peter N. Kazembe,[5] Hamish Dobbie,[5] Madhu Midathada,[1] and William R. Jarvis[3]

[1]HIV Immunology and Diagnostics Branch, [2]Division of AIDS, STD, and TB Laboratory Research, and [3]Investigation and Prevention Branch, Hospital Infections Program, National Center for Infectious Diseases, and [4]Office of Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia; [5]Lilongwe Central Hospital and Community Health Sciences Unit, Ministry of Health and Population, Lilongwe, Malawi

NK cells, γδ T cell antigen receptor chain–positive cells, and $CD3^+CD16/56^+$ (natural T [NT]) cells are involved in innate immunity and immunoregulation; however, their role in clinical infection is not well defined. Cytofluorometric analysis was used to examine peripheral blood from bacteremic, nonbacteremic, and healthy human immunodeficiency virus (HIV)–positive and –negative persons in Malawi, Africa. Mycobacteremia was associated with a higher proportion of $CD3^+CD8^-$ γδ cells (median, 16.6% vs. 0.7% for all other cells; $P < .001$), and *Salmonella* bacteremia was associated with a higher proportion of NT cells (4.3% vs. 2.2%; $P = .002$). HIV plasma RNA levels were weakly positively correlated with NT cells ($r_s = .39$; $P = .002$), NK cells ($r_s = .38$; $P = .003$), and γδ cells ($r_s = .43$; $P < .001$). Compared with patients who survived, patients who died had a higher percentage of NT cells (3.7% vs. 1.9%; $P = .017$) and a higher percentage of NT cells that spontaneously produced interferon-γ (2.4% vs. 1.2%; $P = .035$). The data support the clinical relevance of γδ and NT cells in mycobacterial, *Salmonella,* and HIV infections and of NT cells in mortality.

Several cell types involved in innate immunity play important roles in antimicrobial activity and in the immunoregulation of cells involved in acquired immunity. NK cells were among the first of these cell types to be examined extensively in humans, especially in relation to human immunodeficiency virus (HIV) infection [1–3]. NK are $CD3^-$ lymphocytes with a distinctive histology, function, mechanism of action, and phenotype (e.g., expression of CD16 and/or CD56) [4, 5].

Natural T (NT; sometimes referred to as NKT in the literature) cells, a second immunoregulatory cell type involved in innate immunity, express CD3 and are, therefore, T lymphocytes. NT cells have been reported to express various NK markers, but these markers have varied among the laboratories that investigate NT cells. In this report, NT cells will refer to $CD3^+$ lymphocytes that co-express the classic NK marker CD16 and/or CD56.

We reported elsewhere that NT cells may be involved in HIV-related mortality [6]. Specifically, in a study of bloodstream infections (BSIs) in febrile, hospitalized Thai patients, we found that having a higher proportion of these cells that produce tumor necrosis factor (TNF)–α, a type 1 proinflammatory cytokine, was associated with lower mortality, but only in patients with palpable cervical lymphadenopathy and nonacute HIV infection [6].

Many researchers are investigating a third, relatively small, population of T cells, referred to as γδ cells, that are involved in innate immunity. Like other T cells, γδ cells are coupled to the antigen-independent CD3 molecule, but they have γδ T cell antigen–receptor chains, rather than αβ chains, that form the antigen-specific portion of the T cell–antigen receptor. γδ T cells have been studied in relation to various infections [7], including HIV [8, 9], mycobacterial infections [10–12], and bacterial infections, such as *Salmonella* [13, 14].

The NK, NT, and γδ lymphocyte populations share certain characteristics, and some human γδ cells express a variety of NK receptors [15]; however, their relationships to one another have not been defined. All 3 lymphocytes are non–major histocompatibility complex (MHC) restricted and can recognize nonpeptide antigens [4, 5, 7, 9, 16, 17]. All are quantitatively small cell populations in the peripheral blood, are normally present in all humans, are involved in innate immunity, and appear to have important immunoregulatory properties, es-

Received 21 December 1999; revised 1 May 2000; electronically published 18 July 2000.

The study protocol was approved by the institutional review boards of the Centers for Disease Control and Prevention and Lilongwe Central Hospital. Informed consent was obtained from all study participants.

Use of any and all trade names and commercial sources is for identification only and does not imply endorsement by the Public Health Service or the US Department of Health and Human Services.

Reprints or correspondence: Dr. Janine M. Jason, Immunology Branch, Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases, Mailstop A-25, CDC, 1600 Clifton Rd. NE, Atlanta, GA 30333 (JMJ1@CDC.gov).

The Journal of Infectious Diseases 2000; 182:474–81

0022-1899/2000/18202-0013$02.00

Table 1. Characteristics of immune study participants, by type of bloodstream infection (BSI).

Characteristic	MB (n = 10)	*Salmonella* BSI (n = 13)	Other BSI (n = 46)	BC$^-$ (n = 97)	Controls[a] (n = 62)
Male[b]	78	46	42	45	NO
Median age, years (range)[c]	37 (22–43)	34 (22–48)	30 (14–61)	28 (13–60)	NO
HIV-positive [d]	100	92	62	71	40
Mortality[e]	33	44	16	13	0

NOTE. Data are %, except as noted for age. MB, mycobacteremia; BC$^-$, blood culture results negative for BSI; NO, not obtained; HIV, human immunodeficiency virus.

[a] One control subject was excluded for technical reasons.

[b] Sex was not recorded for 4 patients, including 1 in mycobacteremia, 1 in other BSI, and 2 in BC$^-$ groups.

[c] Age was not recorded for 6 patients, including 1 in mycobacteremia, 1 in other BSI, and 4 in BC$^-$ groups.

[d] HIV status was unknown for 1 patient and 22 controls.

[e] Outcome was unknown for 24 patients.

pecially in relation to antimicrobial immunity and autoimmunity. For all 3 lymphocytes, immunoregulation appears to occur secondary to fluctuations in their production of type 1 and/or type 2 cytokines under various conditions. These cytokine patterns influence the development, cytokine production, and activity of nearby peptide antigen–specific immune cells.

To further investigate the roles of these 3 lymphocyte types in antimicrobial immunity and to expand our previous investigation in Thailand, we evaluated adults admitted to a community hospital in Lilongwe, Malawi. As in Thailand, the evaluations were done as part of a study to assess the incidence and causes of BSIs in persons in developing countries. In particular, we examined the relationships of NK, NT, and γδ cells with mycobacterial and *Salmonella* BSIs and with HIV. We also explored whether any relationship exists between NT and γδ cells—2 T cells that serve similar functions.

Methods

Patients. At the Lilongwe Central Hospital, 471 febrile (oral temperature, 38°C), hospitalized patients who were 13–64 years old (median, 30 years) were enrolled in a study to assess the incidence of BSIs. Half these patients were male, 73% were positive for HIV antibody, and 28% had a blood culture result positive for a BSI.

For each febrile participant, HIV antibody testing was done at the hospital, a medical history was obtained, and a physical examination was done by one of the investigators. For a random subset of 166 of these 471 patients and for 62 afebrile, asymptomatic local volunteers (controls; table 1), measurements of various immune parameters were determined by flow cytometry at admission or during the same time period that patient samples were obtained. These measurements included assessment of intracellular cytokines and percentages of lymphocytes that expressed γδ, CD3, CD8, and/or CD16/CD56. HIV antibody testing was done for 40 of the 62 controls.

As in most developing nations, HIV-infected persons in this study neither were receiving antiviral therapies nor were being monitored for changes in CD4 cell counts or HIV plasma RNA levels. However, we determined HIV-1 plasma RNA levels for 48 patients and 13 controls at the Centers for Disease Control and Prevention (CDC) by use of a Roche Monitor (version 1.5; Roche Diagnostics, Indianapolis), which has a lower detection limit of 400 copies/mL. Of the 48 patients, 26 had a BSI (mycobacteria, 1; *Salmonella,* 11; other BSIs, 14) and 22 were febrile and had blood culture results negative for a BSI.

Laboratory procedures. Blood samples were cultured, as described elsewhere [6]. Plates were incubated at 35°C for 7 days, and the cultures were examined each day during incubation. These culture techniques readily detect pathogenic bacteria, fungi, and mycobacteria species.

Blood was prepared for cytokine stimulation, as described elsewhere [6]. For induced cytokine expression, blood was stimulated for 5 h at 37°C with 200 ng/mL phorbol 12-myristate 13-acetate (PMA) and 4 μg/mL ionomycin (both from Sigma, St. Louis) in the presence of 40 μg/mL brefeldin-A (Sigma) and RPMI 1640 with L-glutamine. For spontaneous cytokine expression, blood was incubated under the conditions noted above, but the media lacked PMA and ionomycin. After being washed, the red blood cells were lysed with ammonium chloride solution and then lymphocytes were permeabilized and fixed (Ortho Permeafix; Ortho Diagnostic Systems, Raritan, NJ). After being processed, the samples were shipped at 4°–8°C to the CDC for further analysis. For standard surface phenotypic markers (e.g., CD4, CD45RO), values for unstimulated cells are used herein.

Flow-cytometry reagents. Fluorescein isothiocyanate (FITC)–conjugated, phycoerythrin (PE)–conjugated, PE–cyanine 5 (PE-Cy5), peridinin chlorophyll protein (PerCP)–conjugated, and allophycocyanin (APC)–conjugated murine monoclonal antibodies were obtained from the following sources: Becton Dickinson Immunocytometry Systems/PharMingen (BD), San Jose, CA (CD3, CD4, CD8, CD45RO, CD16, CD56, interleukin [IL]–4); Immune Source, Reno (interferon [IFN]–γ, TNF-α); CalTag Laboratories, Burlingame, CA (CD8); and Endogen, Woburn, MA (T cell antigen receptor chains γδ, δ1, and δ2). Isotype controls were obtained from BD. All staining was done after permeabilization to detect both surface and cytoplasmic antigens. The antigens assessed in this study provided reliable results with staining, when the permeabilization protocol was followed (data not shown).

Peripheral blood mononuclear cells from study participants were stained with 1 of the 2 following combinations: (1) T cell receptor

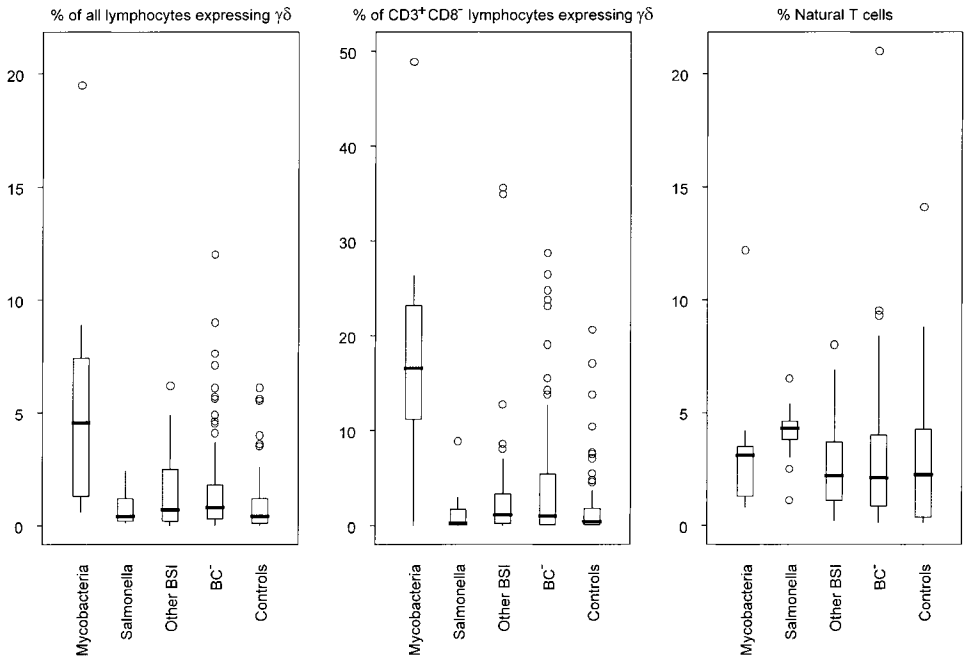

Figure 1. γδ and natural T cell parameters that were significantly related to the type of bloodstream infection (BSI). *Boxes* indicate values between the 25th and 75th percentiles; *horizontal lines* represent medians; *vertical lines* are the farthest values within $\pm 1.5 \times$ the interquartile range from the 25th and 75th percentiles; *circles* represent outliers. BC^-, blood culture results negative for BSIs. Note that scales differ among panels.

γδ–FITC, CD14-PE, CD8–PE-Cy5 or PerCP, and CD3-APC or (2) TNF-α–FITC, CD16/56-PE, CD3–PE-Cy5 or PerCP, and INF-γ–APC. In addition, the γδ T cell population of 9 individuals (4 HIV-positive patients with mycobacteremia [MB] and 5 patients [3 HIV-positive] who had BSI-negative blood culture results) for whom stored cells were available was further assessed, by using various combinations of δ1 or δ2-FITC, IL-4–PE, CD16/56-PE, CD3-PerCP, IFN-γ–APC, and CD45RO-APC.

Flow cytometry. Samples were stained after shipment to the CDC. Staining was done at room temperature for 30 min in the dark. Four-color flow cytometry (FACSort or FACSCalibur; BD) was done by using CellQuest software (BD). Between 50,000 and 80,000 events were collected from each tube in the panel. Lymphocytes were defined on the basis of forward and side light scatter.

Analytic techniques. Analyses included data for all lymphocytes, including $CD3^+$ cells, $CD3^+CD8^+$ cells, $CD3^+CD8^-$ cells, $CD3^+CD16/56^+$ cells (NT), $CD3^-CD16/56^+$ cells (NK), and $\gamma\delta^+$ lymphocytes. Within the $\gamma\delta^+$ population, analyses included data for the $CD8^+$ and $CD8^-$ cell subpopulations. For stored cells that were analyzed at a later time, $\delta1^+$ and/or $\delta2^+$ lymphocytes, the 2 alleles of the δ chain, were examined.

Statistical techniques. Dichotomized subgroups were compared by using the Wilcoxon rank sum and signed rank tests and Spearman correlation coefficients. Proportions were compared by using Fisher's exact or Pearson's χ^2 tests. Data analyses were done by using data for all participants and were repeated for HIV-positive participants, since all the participants with MB were HIV positive. To better differentiate the effects of HIV infection and "other BSIs," we performed logistic regression analyses, while controlling for HIV disease severity, and compared parameter values between various pairs of BSI subgroups (patients with MB, patients with *Salmonella* bacteremia, patients with other BSIs, patients with blood cultures negative for BSIs, and controls) and between patients who survived and those who died. For these analyses, CD4 cell percents (CD4%) or HIV plasma RNA levels were used as an independent variable indicative of HIV disease severity; thus, the logistic regression significance level for each immune variable indicates its significance, while controlling for the effects of HIV disease severity.

For analyses with outcome as the dependent variable, we also used BSI group as an independent, 5-category variable; thus, both the CD4% and infection effects were controlled for in these analyses. Spearman's rank correlations (r_s) were computed to assess correlations with plasma levels of HIV RNA and with CD4%; for samples with undetectable levels of RNA, the analysis value was set at half the lower limit of the assay (200 copies/mL). The significance level for all analyses was set at $P < .05$. Box plots were constructed and drawn by using S-PLUS (MathSoft, Seattle).

Results

γδ were associated with MB, and NT was associated with Salmonella *bacteremia.* Participants with MB included 9 patients with *Mycobacterium tuberculosis* and 1 with *M. avium–intracellulare* complex. All patients with MB were also HIV positive. Participants with MB had the lowest median CD4%, which was indicative of more-severe HIV disease (2.6% vs. 5.7%

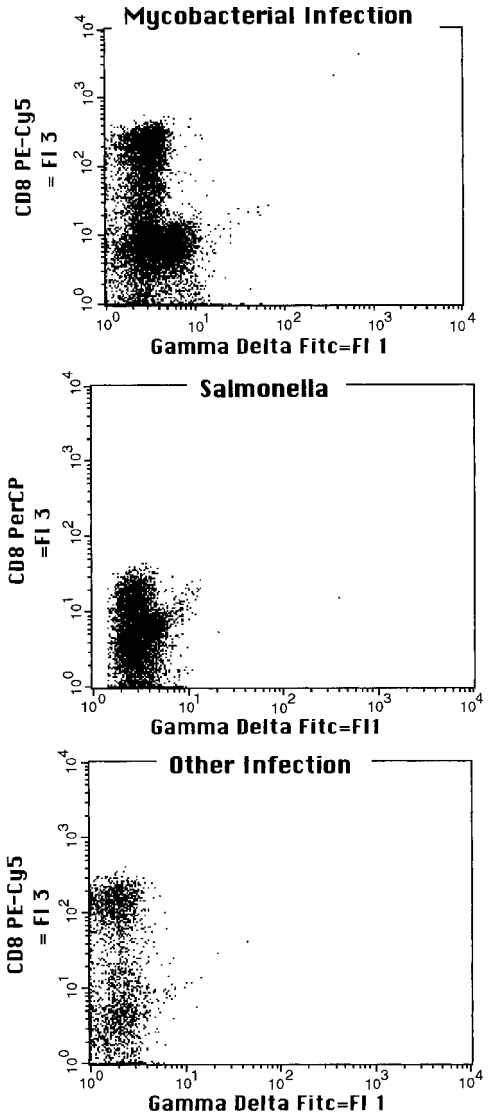

Figure 2. Dot plots of γδ expression by lymphocytes, by type of bloodstream infection. FITC, fluorescein isothiocyanate conjugated; PE, phycoerythrin conjugated; PE-Cy5, phycoerythrin–cyanine 5 conjugated; PerCP, peridinin chlorophyll protein conjugated; Fl 1 and Fl 3, fluorochrome 1 and 3.

for *Salmonella* bacteremia, 15.7% for those with other BSIs, 10.4% for those with BSI-negative blood culture results, and 34.7% for controls). With logistic regression analyses, only the percent of lymphocytes and the percent of $CD3^+CD8^-$ lymphocytes that expressed γδ significantly differentiated the group with MB from all other infection groups (table 2, figure 1). Persons with MB had the highest percentage of these cells (figure 2).

Despite the small number of patients with MB, differences were highly significant, even after we controlled for the effects of HIV disease severity (CD4%) with logistic regression analysis. In later analyses that used stored cells, patients with MB ($n = 3$) had a higher proportion of δ1 T cells than did participants with BSI-negative blood culture results ($n = 4$) (mean, 19.7% vs. 4.8%; median, 15.0% vs. 4.5%; $P < .001$). Patients with MB also had a higher proportion of δ2 cells (mean, 16.3% vs. 5.3%; median, 10.0% vs. 5.5%; $P = .008$).

Subjects with *Salmonella* bacteremia had the highest percentage of NT (table 2 and figure 1); this was true for those who survived and those who died. Logistic regression analysis for the percentage of NT cells could not be completed between and participants with MB and those with *Salmonella* bacteremia but was significant between those with *Salmonella* bacteremia and those with other BSIs and between those with *Salmonella* bacteremia and the control participants, again even with the effect of HIV disease severity taken into account.

NT were strongly associated with mortality. The percentage of lymphocytes that were NT cells was highly variable, ranging from 0.1% to as high as 21%. Patients who died had a higher

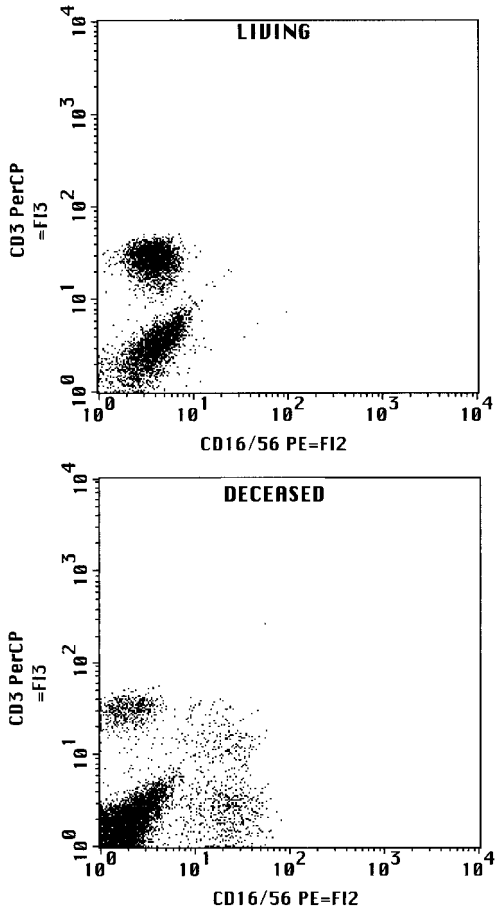

Figure 3. Dot plots of CD16/56 expression by lymphocytes, by mortality status of the subjects. *Right upper quadrant* represents $CD3^+CD16/56^+$ (natural T) cells. PerCP, peridinin chlorophyll protein conjugated; PE, phycoerythrin conjugated; Fl 2 and Fl 3, fluorochrome 2 and 3.

Table 2. γδ and natural T (NT) cell parameters significantly related to type and presence of bloodstream infection (BSI) in logistic regression analyses.

Variable, patient group	Median	P[a] vs. Patients with MB	P[a] vs. Patients with SB
Percentage of all lymphocytes that express γδ			
Patients with MB (n = 10)	4.6	Referent	.002[b]
Patients with SB (n = 13)	0.4	.002[b]	Referent
Patients with other BSIs (n = 46)	0.7	.001[b]	NS
Patients with negative blood culture results (n = 97)	0.8	.002[b]	NS
Controls (n = 61)	0.4	<.001[b,c]	NS
Percentage of $CD3^+CD8^-$ lymphocytes that express γδ			
Patients with MB	16.6	Referent	.001[b]
Patients with SB	0.2	.001[b]	Referent
Patients with other BSIs	1.1	<.001[b]	NS
Patients with negative blood culture results	1.0	<.001[b,c]	NS
Controls	0.4	<.001[b]	NS
Percentage of lymphocytes that express CD3 and CD16/56 (NT)			
Patients with MB	3.1	Referent	.054[d]
Patients with SB	4.3	.054[d]	Referent
Patients with other BSIs	2.2	NS	.002[b]
Patients with negative blood culture results	2.1	NS	.004
Controls	2.3	NS	.017[b]

NOTE. MB, mycobacteremia; SB, *Salmonella* bacteremia; NS, not significant.

[a] By Wilcoxon rank sum test, comparisons remained significant in a logistic regression analysis, including human immunodeficiency virus disease severity, as represented by CD4%.

[b] CD4%.

[c] Interaction between CD4% and immune parameter.

[d] Logistic regression analysis could not be done because of a complete separation of points.

percentage of NT cells (P = .013; figures 3, 4); this difference remained highly significant in a logistic regression analysis in which we controlled for both HIV disease severity, as represented by CD4%, and infection group (table 3). Also, a higher percentage of NT lymphocytes were spontaneously producing IFN-γ (P = .044) and IFN-γ and TNF-α (P = .025; table 3 and figure 4). The percentage of NT cells with induced cytokine production did not differ significantly between patients who lived and those who died (IFN-γ, 38.9% vs. 35.2%; TNF-α, 27.5% vs. 28.6%; both cytokines, 16.6% vs. 12.1%) and neither did the percentage of NT cells that spontaneously produced TNF-α (2.0% vs. 4.0%; P = .103 in univariate testing, P = .072 in logistic regression analysis).

NK, γδ, and NT cells were all weakly correlated with HIV disease severity. For all HIV-positive participants, CD4%, an indicator of HIV disease severity, was significantly negatively correlated with the percentage of lymphocytes that were NK cells (r_s = −0.31; P < .001), the percentage that were γδ cells (r_s = −0.31; P < .001), and the percentage that were $CD3^+CD8^-$ γδ cells (r_s = −0.33; P < .001). HIV disease severity, as represented by CD4%, was positively correlated with the percentage of NK cells induced to produce IFN-γ (r_s = .29; P = .001). Since participants with MB all were HIV positive, with very low CD4%, these patients could have disproportionately skewed the analysis; therefore, they were removed, and the analysis was repeated to assess whether this group biased the statistical result. When this was done, the percentage of NT cells induced to produce TNF-α was also somewhat correlated with HIV disease severity, as indicated by CD4% (r_s = 0.18; P = .048). Plasma levels of HIV RNA were positively correlated with the percent of lymphocytes that were NT cells (r_s = 0.39; P = .002; n = 60), NK cells (r_s = 0.38; P = .003; n = 61), and γδ cells (r_s = 0.43; P < .001; n = 61).

γδ and NT cells are highly overlapping populations (analyses of stored cells). A significantly higher proportion of (but not all) δ1 and δ2 T cells expressed CD16/56 than did all $CD3^+$ lymphocytes from the same patients (n = 6; mean, median [range]: 40.7%, 33% [21%–86%]; 29.5%, 23% [9%–69%]; and 7.7%, 8% [1%–19%], respectively) (P = .008 for both γ1 and γ2 vs. $CD3^+$ lymphocytes). Since δ1 and $\delta2^+$ T cells are included in the value for the entire $CD3^+$ cell population, the value for αβ T cells would be even lower than and more discordant from the δ^+ T cells than the value for the entire $CD3^+$ cell population. The ratio of δ2/δ1 did not vary significantly by HIV status (1.6 vs. 1.3) or by blood culture status (1.6 vs. 1.2). The percentage of cells that expressed CD45RO did not vary significantly among δ1, δ2, and all $CD3^+$ cells (data not shown).

Proportionately more δ1 and δ2 T cells produced type 2 cytokines (stored cells). Many δ1, δ2, and $CD3^+$ lymphocytes could be induced to produce IFN-γ, a type 1 cytokine. The proportion of lymphocytes that produced this cytokine did not differ significantly among these 3 T cell types; however, a small and variable, but significantly higher, proportion of both δ1 and δ2 T cells produced IL-4 than did all $CD3^+$ cells (n = 7, mean, median [range]: 3.1%, 1% [0%–10%]; 4.1%, 1% [1%–8%]; and 0.7%, 0% [0%–2%], respectively) (P = .016 for γ1 and

Table 3. Natural T (NT) cell parameters significantly related to mortality status, in logistic regression analyses.

Parameter	Patients who survived (n = 117)	Patients who died (n = 24)	P[a]	β Estimate	95% CL
Percentage of NT[b]	1.9	3.7	.006	−.327	−.576, −.108
Percentage of NT that spontaneously produce cytokines					
IFN-γ[c]	1.2[d]	2.4	.041	−.138	−.273, −.002
TNF-α and IFN-γ[e]	0.3[f]	0.5	.001	−.540	−.884, −.223

NOTE. CL, confidence limits; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor–α.

[a] Initial logistic regression analyses included parameter, severity of disease (CD4%), and a variable for infection category. Variables that did not contribute significantly to model were removed before final analyses.

[b] For percentage of NT, CD4% contributed to the model (P = .019; β estimate, .081; 95% CI, .022–.160) but infection group did not.

[c] For percentage of NT that produced IFN-γ, the contributions were similar.

[d] n = 116.

[e] For the percentage of NT that produce IFN-γ and TNF-α, infection group contributed (P = .008; β estimate, .466; CI, .118–.811) but CD4% did not.

[f] n = 115.

P = .031 for γ2 vs. $CD3^+$ lymphocytes). The cells that produced IL-4 did not produce IFN-γ; hence, their cytokine profile was type 2 not type 0 (figure 5).

Discussion

NT, γδ, and NK cells all are involved in innate immunity and have a less-specific immune recognition system than the elegant one delineated for αβ T cells [18, 19]. They are non-MHC–restricted and can respond to nonpeptide and glycolipid antigens in the context of CD1 or other non-MHC molecules [16, 20–23]. Much of their immunoregulatory function appears to be cytokine based, and it has been suggested that modifying the cytokine profile of these cells could produce alterations in the entire immune profile. For example, it was reported recently that murine NT cells can be induced by glycolipid activation to make IL-4 and then to modulate other T cells toward a type 2 cytokine profile [24]. These cells might be particularly important in infections that occur in immunosuppressed individuals, in whom antigen-specific immunity is compromised.

Human γδ cells express a variety of NK markers, but the phenotypic and functional overlaps between NT and γδ cells have not been well explored [15]. Because these cells appeared to be important in our patient cohort, we further examined the

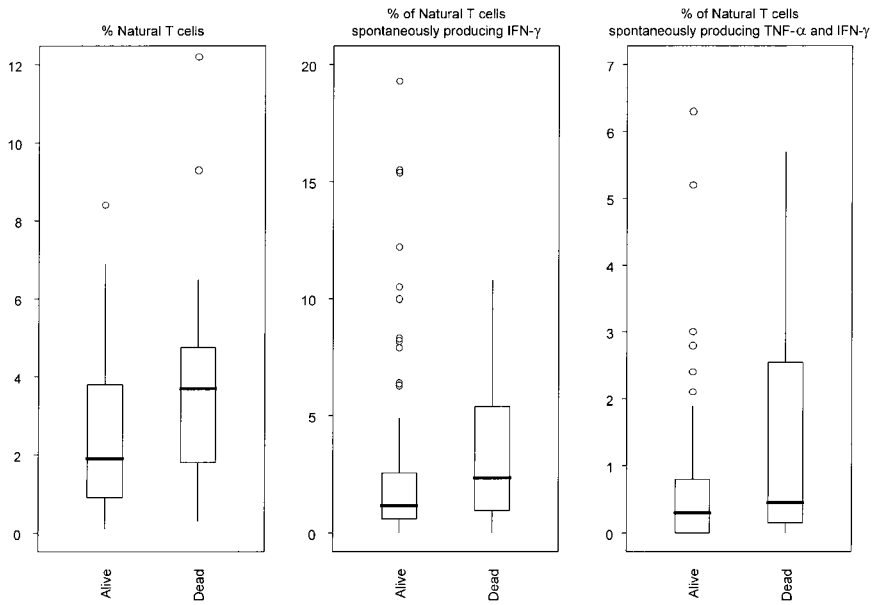

Figure 4. Natural T cell parameters that were significantly related to the mortality status of patients. *Boxes* indicate values between the 25th and 75th percentiles; *horizontal lines* represent medians; *vertical lines* are the farthest values within ±1.5 × the interquartile range from the 25th and 75th percentiles; *circles* represent outliers. Note that scales differ among panels. IFN-γ, interferon-γ; TNF-α, tumor necrosis factor–α.

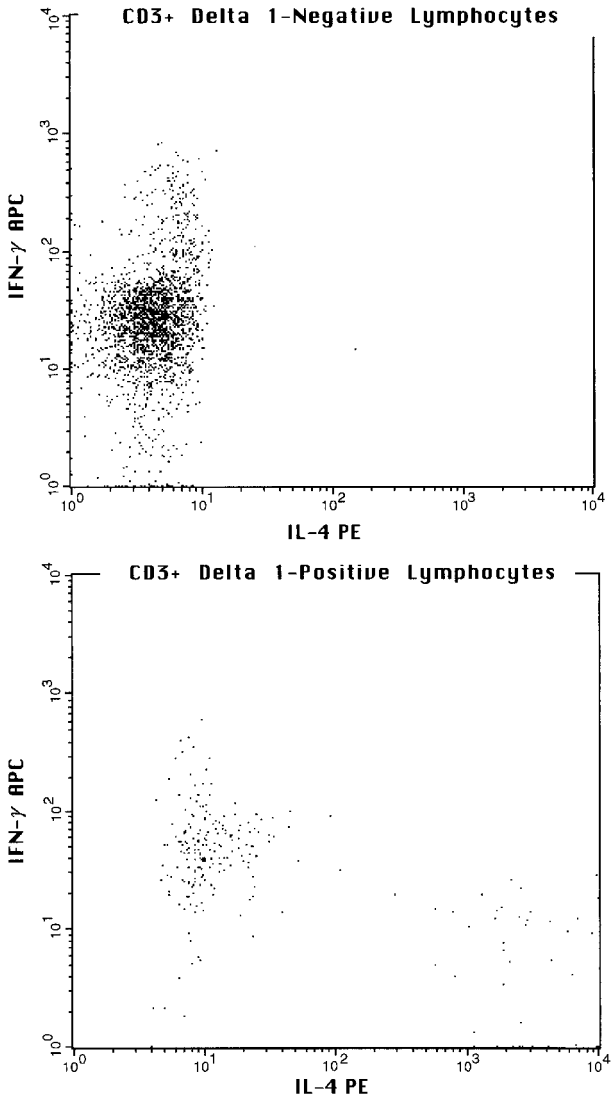

Figure 5. Dot plots of interleukin (IL)–4 and interferon (IFN)–γ production by δ1-negative and δ1-positive T cells. APC, allophycocyanin conjugated; PE, phycoerythrin conjugated.

relationship between them, by using stored cells from Malawi patients. This testing showed that γδ and NT cells were highly overlapping, but by no means identical, populations. Furthermore, consistent with a previous murine study [24], we found that a small, but significantly higher, proportion of γδ cells than all T lymphocytes of these patients produced the type 2 cytokine IL-4 when stimulated in vitro.

An increasing amount of data suggests that NK, γδ, and NT cells may play disproportionately large roles in the regulation of antimicrobial immunity, in particular against mycobacteria, *Salmonella,* and HIV. However, their role in clinical infection is not as well defined as that in murine models and in vitro systems. In studies of pulmonary tuberculosis, the proportions of peripheral blood γδ cells have been described variously as not changing [11, 25], increasing [26], or decreasing (V [variable region]$_{\gamma 9}{}^{+}V_{\delta 2}{}^{+}$) [27]. In one report, the proportion did not change, but these cells were highly activated [11]. With *Salmonella* infection, peripheral blood γδ cells were reportedly increased, and they increased even more with systemic infection ($n = 6$ patients) than with localized gastrointestinal infection ($n = 14$ patients) [13]. NK and γδ cells each has been reported to be involved in HIV disease and disease progression [1–3, 8, 9]. We reported elsewhere an inverse relationship between mortality and the percentage of NT cells that produce TNF-α among febrile Thai patients, but this was found only in HIV-infected patients with cervical lymphadenopathy [6].

In the current study, we examined the relationships between the NK, NT, and γδ cell types and the occurrence of MB or *Salmonella* bacteremia, severity of HIV infection, and mortality in a cohort of febrile hospitalized persons in Malawi, Africa. We found 3 relationships with infection. First, the proportion of cells that expressed γδ was significantly increased in patients with disseminated mycobacterial infection, even when we controlled for the severity of associated HIV infection. We did not find a relationship between γδ expression and disseminated *Salmonella* infection. Second, we found a significant association between *Salmonella* infection and the percent of cells that were NT, which perhaps indicates that some portion of the γδ/NT nonoverlapping cell population may be important in this infection. Third, NK, γδ, and NT cells were all weakly associated with HIV disease severity, as represented by CD4% and/or levels of HIV viral RNA, but not with the presence or absence of HIV infection per se.

In regard to mortality, as in our previous Thai study, we found relationships between NT cells and NT cell cytokine production and mortality. In Thailand, we found the inducible production of TNF-α, a proinflammatory type 1 cytokine, by NT cells to be associated with lower mortality, but we found this only in the HIV-infected patients with lymphadenopathy [6]. This finding was not associated with the presence, absence, or type of associated BSI [6]. In the Malawi study, the percentage of peripheral blood cells that were NT cells, the percentage of these NT cells that spontaneously produced IFN-γ, and the percentage of these NT cells that produced both IFN-γ and TNF-α were significantly higher in subjects who died.

Unlike the Thai patients, no Malawi patients had palpable cervical lymphadenopathy (note: the same team performed physical examinations in both countries.) The presence or absence of cervical lymphadenopathy was, thus, a striking difference between our 2 patient groups. Of interest, 16 cases of bilateral cervical lymphadenopathy caused by rapidly growing mycobacteria were reported recently from a hospital in northeastern Thailand [28]. It is quite possible that these or similar pathogens could have caused the lymphadenopathy seen in our Thai patients, all of whom were immunosuppressed by HIV infection. Diagnoses in the reported cases were all based on

results of lymph node biopsies [28], which could not be done within the context of our study or by our Thai collaborators.

In a different but relevant study, lymphatic inoculation of TNF-α was shown to cause lymphocyte retention in lymph nodes, decreases in peripheral blood γδ cells, and a differential retention of these γδ cells within the TNF-α–stimulated nodes [29]. We hypothesize that in our Thai patients, NT cell production of TNF-α was associated with a physiologically useful relocation of peripheral γδ cells to the site of lymph node–localized mycobacterial infections. We suggest that, in the Malawi cohort, NT cells were also of clinical importance, but in this case they acted deleteriously. While these are merely hypotheses, our data do support the importance of NT cells in the clinical outcome of these febrile patients, many of whom were immunosuppressed by HIV infection. Thus, our Malawi findings support the hypothesis that NT cells may be involved in survival and suggest that the cytokine profile of NT cells may determine whether their role is protective or destructive.

Acknowledgments

We thank Celeste M. McKnight, Rachel M. Addison, and Terry Byrne (Duke University Medical Center) for their technical assistance in the microbiology laboratory. Most of all, we would like to thank the nursing and medical staff of Lilongwe Central Hospital and the patients who participated in this study.

References

1. Fehniger TA, Herbein G, Yu H, et al. Natural killer cells from HIV-1$^+$ patients produce C-C chemokines and inhibit HIV-1 infection. J Immunol **1998**; 161:6433–8.
2. Plaeger-Marshall S, Spina CA, Giorgi JV, et al. Alterations in cytotoxic and phenotypic subsets of natural killer cells in acquired immune deficiency syndrome (AIDS). J Clin Immunol **1987**; 7:16–23.
3. Ullum H, Cozi LA, Aladdin H, et al. Natural immunity and HIV disease progression. AIDS **1999**; 13:557–63.
4. Yoneda K, Osaki T, Yamamoto T. IL-2 signaling in T and natural killer (NK) cells associated with their class I–non-restricted killing activity. Clin Exp Immunol **1996**; 106:179–86.
5. Horwitz DA, Gray JD, Ohtsuka K, Hirokawa M, Takahashi T. The immunoregulatory effects of NK cells: the role of TGF-β and implications for autoimmunity. Immunol Today **1997**; 18:538–42.
6. Jason J, Archibald L, McDonald LC, et al. Immune determinants of organism and outcome in febrile hospitalized Thai patients with bloodstream infections. Clin Diagn Lab Immunol **1999**; 6:73–8.
7. Salerno A, Dieli F. Role of γδ T lymphocytes in immune response in humans and mice. Crit Rev Immunol **1998**; 18:327–57.
8. Rossol R, Dobmeyer JM, Dobmeyer TS, et al. Increase in Vδ1$^+$γδT cells in the peripheral blood and bone marrow as a selective feature of HIV-1 but not other virus infections. Br J Haematol **1998**; 100:728–34.
9. Poccia F, Wallace M, Colizzi V, Malkovsky M. Possible protective and pathogenic roles of γδ T lymphocytes in HIV-infections. Int J Mol Med **1998**; 1: 409–13.
10. Barnes PF, Grisso CL, Abrams JS, Band H, Rea TH, Modlin RL. γδ T lymphocytes in human tuberculosis. J Infect Dis **1992**; 165:506–12.
11. Behr-Perst SI, Munk ME, Schaberg T, Ulrichs T, Schultz RJ, Kaufmann SHE. Phenotypically activated γδ T lymphocytes in the peripheral blood of patients with tuberculosis. J Infect Dis **1999**; 180:141–9.
12. Tsukaguchi K, de Lange B, Boom WH. Differential regulation of IFN-γ, TNF-α, and IL-10 production by CD4(+) αβTCR$^+$ T cells and Vδ2(+)γδ T cells in response to monocytes infected with *Mycobacterium tuberculosis*–H37Ra. Cell Immunol **1999**; 194:12–20.
13. Hara T, Mizuno Y, Takaki K, et al. Predominant activation and expansion of Vγ9-bearing γδ T cells in vivo as well as in vitro in *Salmonella* infection. J Clin Invest **1992**; 90:204–10.
14. Chervenak KA, Lederman MM, Boom WH. Bacterial antigen activation of Vδ1 and Vδ2 γδT cells of persons infected with human immunodeficiency virus type 1. J Infect Dis **1997**; 175:429–33.
15. Battistini L, Borsellino G, Sawicki G, et al. Phenotypic and cytokine analysis of human peripheral blood γδ T cells expressing NK cell receptors. J Immunol **1997**; 159:3723–30.
16. Bendelac A, Rivera MN, Park SH, Roark JH. Mouse CD1-specific NK1 cells: development, specificity, and function. Annu Rev Immunol **1997**; 15:535–62.
17. Leite-de-Moraes MC, Dy M. Natural killer T cells: a potent cytokine-producing cell population. Eur Cytokine Netw **1997**; 8:229–37.
18. Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. Science **1990**; 248:705–11.
19. Boulot G, Bentley GA, Karjalainen K, Mariuzza RA. Crystallization and preliminary x-ray diffraction analysis of the β-chain of a T-cell antigen receptor. J Mol Biol **1994**; 235:795–7.
20. Constant P, Davodeau F, Peyrat MA, et al. Stimulation of human γδ T cells by nonpeptidic mycobacterial ligands. Science **1994**; 264:267–70.
21. Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR. Natural and synthetic non-peptide antigens recognized by human γδ T cells. Nature **1995**; 375:155–8.
22. Chang CS, Brossay L, Kronenberg M, Kane KP. The murine nonclassical class I major histocompatibility complex–like CD1.1 molecule protects target cells from lymphokine-activated killer cell cytolysis. J Exp Med **1999**; 189:483–91.
23. Vance RE, Jamieson AM, Raulet DH. Recognition of the class Ib molecule Qa-1(b) by putative activating receptors CD94/NKG2C and CD94/NKG2E on mouse natural killer cells. J Exp Med **1999**; 190:1801–12.
24. Singh N, Seokmann H, Scherer DC, et al. Cutting edge: activation of NK T cells by CD1d and α-galactosylceramide directs conventional T cells to the acquisition of a Th2 phenotype. J Immunol **1999**; 163:2373–7.
25. Uh ST, Ki SY, Lim GI, et al. The T cell receptor subsets of lymphocytes in bronchoalveolar lavage in patients with active pulmonary tuberculosis. Respir Med **1998**; 92:408–14.
26. Ito M, Kojiro N, Ikeda T, Ito T, Funada J, Kokubu T. Increased proportions of peripheral blood γδ T cells in patients with pulmonary tuberculosis. Chest **1992**; 102:195–7.
27. Li B, Rossman MD, Imir T, et al. Disease-specific changes in γδ T cell repertoire and function in patients with pulmonary tuberculosis. J Immunol **1996**; 157:4222–9.
28. Chetchotisakd P, Mootsikapun P, Anunnatsiri S, et al. Disseminated infection due to rapidly growing mycobacteria in immunocompetent hosts presenting with chronic lymphadenopathy: a previously unrecognized clinical entity. Clin Infect Dis **2000**; 30:29–34.
29. Young AJ, Seabrook TJ, Marston WL, Dudler L, Hay JB. A role for lymphatic endothelium in the sequestration of recirculating gamma delta T cells in TNF-alpha–stimulated lymph nodes. Eur J Immunol **2000**; 30: 327–34.