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Immune Determinants of Organism and Outcome in Febrile Hospitalized Thai Patients with Bloodstream Infections

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Opportunistic infections (OI) and the human immunodeficiency virus (HIV) cause significant morbidity and mortality in developing countries. Immune cell and cytokine profiles may be related to the type and course of OI and to the OI-HIV interaction. Examining cell-specific cytokine production ex vivo has only recently become feasible. In Thailand, 53 febrile, hospitalized adults were enrolled in a study of the immune correlates of bloodstream infections (BSI). On site, blood cells were stimulated ex vivo. Cell-surface antigens and eight intracellular cytokines were subsequently analyzed using flow cytometry to determine associations with mortality and the organism causing the BSI. By logistic regression analysis, the percentage of $CD3^+$ $CD16/56^+$ cells making tumor necrosis factor alpha (TNF-α) ($P = 0.033$) and the percentage of $CD3^-$ $CD16/56^+$ cells (NK) ($P = 0.032$) were related to HIV positivity. Lymph node enlargement with HIV infection and the percentage of $CD3^+$ $CD16/56^+$ making TNF-α were predictive of death. A lower percentage of $CD3^+$ $CD8^+$ lymphocytes making interleukin-8 (IL-8) ($P = 0.005$), fewer monocytes expressing CD14 ($P = 0.009$), and the percentage of $CD3^+$ $CD8^+$ cells producing gamma interferon ($P = 0.011$) were associated with blood culture positivity and the causative organism. For every one point decrease in the percentage of $CD3^+$ $CD8^+$ cells making IL-8, the likelihood of a positive culture increased 23%; for every one point decrease in the percentage of monocytes expressing CD14, the likelihood of a positive culture increased by 5%. Only a few immune cell types and three of their related cytokines were significantly associated with HIV disease outcome or the BSI organism. These cell types did not include $CD3^+$ $CD8^-$ cells (a surrogate for $CD4^+$ cells), nor did they involve cytokines associated with a type I to type II cytokine shift, which might occur with advancing HIV infection. These associations support the premise that $CD8^+$ and $CD16/56^+$ lymphocytes play significant roles in HIV and type I infections.

Opportunistic infections (OI) are major causes of morbidity and mortality in human immunodeficiency virus (HIV)-infected persons. The interaction between OI and HIV may be a critical factor in disease pathogenesis and outcome. For example, in the United States, the death rates from nontuberculous mycobacteria and cryptococcoses are, respectively, 18.5- and 4.3-fold higher than predicted, secondary to the HIV epidemic (26). It has been postulated that cytokines are important determinants of outcome for infectious diseases and, particularly, for the interaction between HIV and OI (21).

Various immune cells can produce one of at least three patterns of cytokines (7, 10, 20). The type I pattern is associated with the production of interleukin-2 (IL-2, tumor necrosis factor alpha (TNF-α), TNF-β, and gamma interferon (IFN-γ); it is induced by IL-12 and potentiates cellular and/or cytotoxic immunity. The type II pattern is associated with IL-4, IL-6, and IL-10 and supports humoral immunity in response to bacterial, some viral, and some parasitic infections (21). The type 0 pattern represents a mixed response pattern. Animal studies support the premise that cytokine profiles are related to the occurrence and outcome of infection for a number of intracellular and extracellular pathogens (7). Whether this holds true in humans has not been as well investigated. Minimal and sometimes conflicting data broadly suggest that a type I response leads to granuloma formation in mycobacterial infection and clearing of trypanosomes; a type II or a mixed pattern may be associated with symptomatic or disseminated mycobacterial disease (7, 22, 23, 30). Cytokine profiles and HIV infection have been investigated but conclusions vary (5, 10, 18). Certain OI, especially mycobacterial and fungal, potentiate HIV disease; however, the cytokine determinants of this effect are not clear (8, 9, 23, 30). Previous studies of cytokines in humans have generally assessed cloned cells or extracellular, i.e., pleural fluid or plasma, cytokines. These may not accurately reflect the immune profiles of the in vivo microenvironment in which cytokines work. Data are also conflicting concerning the relative importance of various immune cells, including natural killer (NK) cells, cytotoxic T lymphocytes, and monocytes, in these same infections in humans (2–4, 6, 12, 13, 15, 17, 22). The study described herein is the first to assess intracellular cytokine profiles and surface molecules in peripheral blood cells of patients with acute bloodstream infections (BSI).

We have adapted recently developed flow cytometric techniques to evaluate immune cell-surface antigens and intracellular cytokines in patients at field settings in developing countries (reference 11 and unpublished data). For the present study, we used these adapted techniques to assess a random sample of febrile patients at one hospital in Thailand. Immune parameters were assessed for blood drawn at the time of blood

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TABLE 1. Analysis panel for flow cytometric evaluation of surface and cytoplasmic antigens[a]

Tube no.	Cytokine or cell antigen specificity of MAb, with the following conjugate:				
	FITC	PE	PerCP	PECy5	APC
1	CD45	CD8	CD3		CD4
2	CD1c	CD14	CD3		CD19
3	IL-12	IL-4	CD3		CD19
4	CD69	IL-10	CD3		
5	Anti-MT	IL-8		CD8	CD3
6	TCR γδ	TNF-β		CD8	CD3
7	CD8	IL-6	CD3		IFN-γ
8	TNF-α	CD16/56	CD3		IFN-γ

[a] Anti-MT, anti-microtubulin; TCR, T-cell-antigen receptor.

culture. We hypothesized that certain parameters, especially certain intracellular cytokines and/or cytokine patterns, might be predictive of one or both of the following: (i) survival and (ii) the organisms cultured from the blood. We included in our analyses key surface antigens defining various cell populations: a spectrum of type I and type II cytokines, in combinations permitting differentiation of type 0 cells producing both types of cytokines; regulatory cytokines such as IL-12; and surface molecules associated with the immune responses to mycobacterial and bacterial infections (CD14 and CD1) (Table 1).

MATERIALS AND METHODS

Patients. At the Bamrasnaradura Infectious Diseases Hospital, Nonthaburi, Thailand, 246 consecutive febrile (oral temperature, ≥38°C), hospitalized adult patients were enrolled between 12 February and 4 April 1997 in a study to assess the causes of BSI in developing countries. Of these participants, a random sample of 53 individuals also had immune studies done, as described below. This subset was comparable to the entire study population (Table 2). Several members of the subset were recruited each day throughout the entire course of the study. The study protocol was approved by the Centers for Disease Control and Prevention (CDC) and hospital institutional review boards, and informed consent was obtained from all patients. Complete blood count and differential and HIV antibody testing were done at the hospital, a medical history was obtained, and a physical examination was performed by one of the investigators (L.A., C.M., or S.R.). (As in most, if not all, developing countries at this time, HIV-infected persons in this study were not receiving antiviral therapies nor being monitored for CD4 counts or HIV viral titers; these capabilities were not available at this Thai hospital or in this area of Thailand at the time of this study.) Clinical data analyzed included HIV antibody status; acute and chronic symptoms of cough, fever, chills, and diarrhea; respiratory and heart rates on admission; and the clinical presence or absence on admission of candidiasis, oral leukoplakia, Kaposi's sarcoma, lymph node enlargement (LNE, defined as cervical lymph nodes being palpable bilaterally), hepatosplenomegaly, pulmonary symptoms, and a *Mycobacterium bovis* BCG scar. Each patient enrolled in the study was monitored daily as an inpatient, and their clinical course and outcome (survival or mortality) were recorded.

Laboratory procedures. (i) Blood cultures. Blood cultures were performed as previously described (1). The commercially prepared biphasic (broth-agar) systems Septi-Chek and Myco-Chek (Becton Dickinson Microbiology Systems Sparks, Md.) were used for aerobic bacterial and mycobacterial blood cultures, respectively. Briefly, 20 ml of blood was obtained and 10 ml was inoculated into a Septi-Chek bottle at the bedside and 10 ml was inoculated into a lysis centrifugation tube (Isolator; Wampole Laboratories, Cranberry, N.J.) system from which the pellet was inoculated onto standard medium. All cultures were incubated at 35°C and examined daily for 7 days, and organisms were identified to the species level by standard methods. These culture techniques have been shown previously to detect nearly all pathogenic bacteria, fungi, and mycobacterium species (31).

(ii) Cytokine stimulation. Reagents and concentrations used included phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical Co., St. Louis, Mo.), 200 ng/ml; brefeldin A (BFA) (Sigma), 40 μg/ml; ionomycin (Sigma), 4 μg/ml; and ORTHO PermeaFix (ORTHO Diagnostics, Inc., Raritan, N.J.). Two milliliters of blood was collected in heparinized vacutainers and used directly in a whole-blood assay. Blood was stimulated for 5 h with PMA and ionomycin in the presence of BFA and two ml of RPMI 1640 with 2 mM L-glutamine. ORTHO PermeaFix was then added, followed by a buffered saline wash; all tubes were shipped at 4 to 8°C to the CDC for further analyses, which were done within 2 weeks. Stimulation was done because extensive published data, including our own, indicate a general lack of detectable intracellular cytokines in unstimulated human periphiperal blood mononuclear cells (11, 24).

Flow cytometric reagents. Fluorescein isothiocyanate (FITC)-conjugated, phycoerythrin (PE) conjugated, PE-cyanine 5 (PECy5)-conjugated, peridinin chlorophyll protein (Per CP)-conjugated, or allophycocyanin (APC)-conjugated, murine monoclonal antibodies (MAb) (Table 1) were obtained from (i) Becton Dickinson (BD) Immunocytometry Systems, San Jose, Calif. (CD8-FITC and -PE [clone SK1], CD3-PerCP and -APC [clone SK7], CD4-APC [clone SK3], CD45-FITC [clone 2D1], CD19-APC [clone SJ25C1], CD14-PE [clone M0P9], CD69 [clone L78], T-cell antigen receptor γδ chains-FITC [clone 11F2], CD16-PE [clone B73.1], and CD56 [clone MY31]); (ii) PharMingen, San Diego, Calif. (CD8-PECy5 [clone RPA-T8], IL-4-PE [clone 8D4-8], IL-8-PE [clone G265-8], IL-10-PE [clone JES3-9D7], and TNF-β-PE [clone 359-81-11]); (iii) Research and Diagnostics, Minneapolis, Minn. (IL-6-PE [clone 1927.311]); (iv) Immune Source, Reno, Nev. (CD8-APC [clone KL.12], IL-12-FITC [clone I.A1], TNF-α-FITC [clone DTX.34], and IFN-γ-APC [clone 13.TR]); (v) Seikagaku, Tokyo, Japan (CD1c [clone HLT3-104D]); and (vi) Sigma (microtubulin [clone DM1A] custom conjugated to FITC by CalTag, South San Francisco, Calif.). Isotype controls were obtained from BD. NK cells were defined as lymphocytes negative for CD3 and positive for CD16 and/or CD56; cells positive for both CD3 and CD16 and/or CD56 are referred to herein as $CD3^+$ $CD16/56^+$. All staining was done postpermeabilization, and thus, staining would detect both surface and cytoplasmic CD3.

The surface antigens assessed in this study were ones previously shown in our laboratory to be stable with this permeabilization-fixation protocol (data not shown), i.e., in our laboratory, by using these techniques, comparable results for the above surface-related antigens were obtained when staining was done either pre- or postpermeabilization. Further, with control samples in our laboratory, these antigen and cytokine proportions remained stable for at least 2 weeks, the time interval between sample collection and analysis in this study (unpublished data). For example, based on seven normal donors, the following results were found at day 0 and week 4 poststimulation, respectively (mean percent ± standard deviation): 47.1% ± 8.5% and 48.8% ± 6.9% of lymphocytes were $CD4^+$, 19.3% ± 5.6% and 17.2% ± 4.0% of lymphocytes were $CD8^+$, 22.1% ± 6.6% and 20.5% ± 6.4% of $CD4^+$ lymphocytes produced IFN-γ, and 53.7% ± 18.8% and 53.5% ± 17.8% of $CD8^+$ lymphocytes produced IFN-γ.

Flow cytofluorometry. All staining was done after permeabilization, fixation, and shipment, at room temperature for 30 min in the dark. Staining was followed by a buffered saline wash. Four-color cytofluorometry was done by using a FACSort cytofluorometer and CellQuest software (BD). From 20,000 to ≥50,000 ungated events were collected from each tube in the panel (Table 1). Lymphocytes were defined on the basis of forward and side scatter, and monocytes were defined on the basis of the forward and side scatter of $CD14^+$ cells (tube no. 2). Isotype controls were used to set quandrants for cytokine assessments.

Analytical and statistical techniques. Analyses were done for all lymphocytes, $CD3^+$ lymphocytes, monocytes, and depending upon the reagent tube (Table 1), $CD3^+$ $CD8^-$ lymphocytes, $CD3^+$ $CD8^+$ lymphocytes, $CD19^+$ lymphocytes, $CD3^+$ $CD16/56^+$ cells and/or NK cells. Downmodulation of some surface receptors routinely occurs with this stimulation protocol, with CD4 downmodulation being greater than that of CD45, CD3, or CD8. These variables were therefore used only for gating and not for analysis. Univariate statistical comparisons were made for various cell populations, comparing findings for various dichotomized dependent variables, as described below. For example, we compared the percentage of $CD3^+$ $CD8^+$ lymphocytes producing IFN-γ in participants who died to the percentage of $CD3^+$ $CD8^+$ lymphocytes producing IFN-γ in participants who survived. Proportions were compared by using Fisher's exact test. Further analyses were done to assess two key dependent variables: clinical outcome (alive versus dead) and cause of BSI. The cause of BSI was analyzed three different ways: (i) any organism isolated versus no organism isolated; (ii) a type I organism isolated versus all other culture results, with type I organism defined as mycobacterium, cryptococcus, or fungus; and (iii) the specific organism isolated versus all other culture results. Analyses were also done assessing LNE and HIV infection as dependent variables. For each dependent variable, univariate analyses were done using Kruskal-Wallis tests to determine which immune parameters, separately, were associated with the dependent variable.

TABLE 2. Characteristics of the entire study population and of the immune substudy population

Variable	Entire population (n = 246)	Immune substudy (n = 53)
% Male	70	74
Age[a]	32 (15–87) yr	30 (18–75) yr
HIV positivity	74%	81%
BSI positivity	48%	55%

[a] Data are median values. Ranges are given in parentheses.

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TABLE 3. Distribution of patients by infecting pathogen and outcome

Diagnosis	Outcome (% [n])	
	Alive	Dead
Type I infection		
M. avium	71.4 (5)	28.6 (2)
M. tuberculosis	60.0 (3)	40.0 (2)
Other acid-fast bacteria	100.0 (1)	0 (0)
Cryptococcus	87.5 (7)	12.5 (1)
Fungus	100.0 (3)	0 (0)
Total	79.2 (19)	20.8 (5)
Bacterial infection		
Gram-positive bacteria	0 (0)	100.0 (1)
Gram-negative bacteria	50.0 (2)	50.0 (2)
Total	40.0 (2)	60.0 (3)
No organism identified	79.2 (19)	20.8 (5)

The immune parameters found to be significant at the 0.1 level were included in a stepwise logistic regression model. All analyses were done using SAS statistical software (Cary, N.C.). Six patients with bacteremia were not included in this analysis because they had polymicrobial infections; these included *Salmonella* spp.-*Mycobacterium avium* (2), *Salmonella* spp.-other acid-fast bacteria (1), *Salmonella* spp.-*Pseudomonas* spp. (1), *Pseudomonas* spp.-*Cryptococcus* spp. (1), and *S. aureus*-*M. avium* (1).

RESULTS

Determinants of clinical outcome: LNE, HIV positivity, and TNF-α production by $CD3^+$ $CD16/56^+$. (i) Clinical correlations. Of the clinical findings evaluated, only LNE was associated with immune findings or clinical outcome. LNE occurred only in HIV-positive participants: 27 of 43 (63%) of HIV-positive participants had LNE compared to 0 of 10 (0%) of HIV-negative participants ($P < 0.001$). For HIV-positive participants, two variables in a logistic regression analysis were significantly related to LNE: the percentage of lymphocytes expressing the B-cell marker CD19 ($P = 0.011$) (for participants with LNE, 9.2% of lymphocytes were $CD19^+$; for participants without LNE, 12.7% of lymphocytes were $CD19^+$) and the percentage of $CD3^+$ $CD16/56^+$ cells making TNF-α ($P = 0.012$) (for participants with LNE, 4.5% of $CD3^+$ $CD16/56^+$ lymphocytes made TNF-α; for participants without LNE, 20.3% of $CD3^+$ $CD16/56^+$ lymphocytes did so).

(ii) Correlates of HIV infection. In a logistic regression analysis, only two variables were significantly associated with HIV positivity: the percentage of $CD3^+$ $CD16/56^+$ cells making TNF-α ($P = 0.033$) (7.0% for HIV-positive participants versus and 25.1% for HIV-negative participants) and the percentage of lymphocytes that were NK ($P = 0.032$) (2.4 versus 0.6% for HIV-positive versus -negative participants, respectively).

(iii) Correlates of outcome. None of 10 HIV-negative patients versus 13 of 43 (30%) HIV-positive patients died ($P = 0.096$). Within the HIV-positive patient group, 11 of 27 (41%) participants with LNE died compared to 2 of 16 (13%) without LNE ($P = 0.086$). The presence of a positive blood culture was not significantly related to mortality among all participants ($P = .712$) (Table 3) or within the HIV-positive subgroup: 8 of 28 (29%) for whom an organism was isolated died versus 5 of 15 (33%) of those with a negative blood culture ($P = 0.742$). No particular pathogen or type of organism (type I organism versus bacteria versus no organism) was associated with increased mortality in a logistic regression model. In univariate analysis, several immune variables were associated with survival (Table 4); however, in logistic regression analysis, no combination was significant. Since the percentage of $CD3^+$ $CD16/56^+$ cells making TNF-α and the variables of LNE, HIV infection, and outcome were all interrelated, we examined these variables in terms of one another. TNF-α production by $CD3^+$ $CD16/56^+$ cells was significantly and inversely related to LNE ($P = 0.001$) and tended also to be inversely related to HIV positivity and survival, although the latter associations did not reach significance (Table 5). This interrelationship was not found for NK cells or all $CD3^+$ lymphocytes.

TABLE 4. Immune parameters significantly associated with survival of HIV-positive participants

Immune parameter by cell population	No. of participants assessed, identified by outcome		% Cell population positive for participants, identified by outcome		
	Alive	Dead	Alive	Dead	P value[a]
All lymphocytes					
% $IL\text{-}6^+$	29	13	6.5	1.0	0.016
% $IFN\text{-}\gamma^+$	30	13	12.1	5.5	0.043
% $IL\text{-}6^+$ $IFN\text{-}\gamma^+$	29	13	0.2	0.1	0.032
$CD3^+$ lymphocytes					
% $IL\text{-}6^+$ $IFN\text{-}\gamma^+$	29	13	0.3	0.1	0.008
$CD3^+$ $CD8^+$ lymphocytes					
% $IFN\text{-}\gamma^+$	29	13	30.0	22.6	0.028
% $IL\text{-}6^+$ $IFN\text{-}\gamma^+$	29	13	0.6	0.2	0.031
$CD3^+$ $CD16/56^+$ lymphocytes					
% $TNF\text{-}\alpha^+$	28	11	13.8	3.8	0.009
% $TNF\text{-}\alpha^+$ $IFN\text{-}\gamma^+$	28	11	8.4	1.9	0.005
Monocytes (by scatter)					
% $CD1c^+$	30	13	12.0	0.7	0.018

[a] P values for comparisons between living and dead participants for described parameters by using Kruskal-Wallis chi square approximation analysis.

Determinants of culture positivity and organism isolated: CD14 expression by monocytes and IL-8 and IFN-γ production by $CD3^+$ $CD8^+$ lymphocytes. Study participants had BSI which were due to a wide variety of pathogens (Table 3). HIV-infected patients were significantly more likely to have a positive blood culture: 28 of 43 (65%) HIV-positive versus 1 of 10 (10%) HIV-negative patients had positive blood cultures ($P = 0.003$). (The one HIV-negative patient with a positive blood culture had a fungal infection). LNE was not associated with blood culture positivity: 16 of 29 (55%) HIV-positive participants with LNE versus 13 of 26 (50%) without LNE had an organism isolated ($P = 0.790$). By univariate analysis, 11 immune variables were significantly associated with the type of organism isolated from the blood (Table 6).

(i) Isolation of any organism. In a logistic regression model, culture positivity was associated with a lower percentage of

TABLE 5. Median percentages of $CD3^+$ $CD16/56^+$ cells producing TNF-α, by patient subgroup

Patient subgroup	No. of patients	% $CD3^+$ $CD16/56^+$ cells producing TNF-α
HIV^-, no LNE, survived	10	25.1
HIV^+, no LNE, survived	14	23.3
HIV^+, LNE, survived	14	5.3
HIV^+, LNE, deceased	10	3.8
HIV^+, no LNE, deceased	1	0

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TABLE 6. Median values for immune parameters, by diagnosis of participants with monomicrobial BSI

Immune parameter by cell population	No. of patients with:			% Cell population positive, by infection status			P value[a]
	Type I infection	Bacterial infection	Negative blood culture	Type I infection	Bacterial infection	Negative blood culture	
All lymphocytes							
% $CD3^-$ NK	24	5	24	2.6	5.1	1.2	0.021
% $IL\text{-}6^+$	23	5	24	3.6	0.2	6.5	0.019
% $IL\text{-}6^+$ $IFN\text{-}\gamma^+$	23	5	24	0.2	0.0	0.2	0.030
% $IL\text{-}10^+$	23	5	24	7.6	0.8	6.9	0.009
$CD3^+$ lymphocytes							
% $IL\text{-}4^+$	24	5	24	3.0	0.2	3.4	0.034
% $IL\text{-}6^+$	23	5	24	1.6	0.3	0.8	0.047
% $IFN\text{-}\gamma^+$	24	5	24	33.6	12.9	17.3	0.005
$CD3^+$ $CD8^+$ lymphocytes							
% $IL\text{-}8^+$	23	5	24	4.3	1.1	7.5	0.004
% $IFN\text{-}\gamma^+$	23	5	24	35.9	10.9	27.0	0.007
$CD19^+$ lymphocytes							
% $IL\text{-}4^+$	21	4	21	0.9	0.0	0.2	0.022
Monocytes (by scatter)							
% $CD14^+$	24	5	24	8.1	4.6	17.3	0.027
% $IL\text{-}4^+$ $IL\text{-}12^+$	24	5	24	0.4	0.0	0.1	0.019
% $TNF\text{-}\alpha^+$	23	5	23	0.4	0.0	0.6	0.004
% $TNF\text{-}\alpha^+$ $IFN\text{-}\gamma^+$	23	5	23	0.2	0.0	0.3	0.008

[a] P values are for three-way comparisons among participants with type I infections, bacterial infections, or negative blood cultures for each described variable. For example, for the percentage of all lymphocytes producing IL-6 in the peripheral blood samples of patients with type I organisms in their bloodstream (3.6%) compared to the percentage of all lymphocytes producing IL-6 in the peripheral blood samples of patients with bacteria in their bloodstream (0.2%) compared to the percentage of all lymphocytes producing IL-6 in the peripheral blood samples of patients with no organism grown from their bloodstream (6.5%), the P value is 0.019 (Kruskal-Wallis chi square approximation analysis).

$CD3^+$ $CD8^+$ lymphocytes making IL-8 ($P = 0.005$ for those with a positive blood culture versus those with a negative blood culture) and fewer monocytes (defined by scatter pattern) expressing CD14 on their surface ($P = 0.009$ for those with a positive blood culture versus those with a negative blood culture). For every one point decrease in the percentage of $CD3^+$ $CD8^+$ cells making IL-8, the likelihood of a positive culture increased 23%; for every decrease by one point in the percentage of monocytes expressing CD14, the likelihood of a positive culture increased by 5%. In the model, culture positivity was also statistically related to the percentage of $CD3^+$ $CD8^+$ cells producing IFN-γ ($P = 0.011$). These same variables remained in the model when HIV-positive patients were analyzed separately.

(ii) Type I infection versus bacterial infection or negative blood culture. In a logistic regression model, type I infection was associated with IL-8 and IFN-γ production by $CD3^+$ $CD8^+$ lymphocytes and CD14 expression on monocytes. The percentages of $CD3^+$ $CD8^+$ cells producing IL-8 and the percentages of monocytes expressing CD14 were highest for patients with negative cultures and lowest for patients with bacterial infections (Table 6). Within the type I group, those with an *M. tuberculosis* infection had the lowest median percentage of monocytes expressing CD14: *M. tuberculosis* 1.5%; *M. avium*, 21.7%; cryptococcus, 8.2%; fungi, 14.1% ($P = 0.043$ for *M. tuberculosis* versus all other culture results). Type I infection was also associated with the highest percentage of $CD3^+$ $CD8^+$ cells producing IFN-γ (Table 6), especially in non-*M. tuberculosis* infections: *M. avium*, 37.1%; other acid-fast bacteria, 100%; *M. tuberculosis* 19.4%; cryptococcus, 35.1%; fungis: 23.9%.

(iii) Bacterial infection versus type I or negative culture. Only five bacterial BSI occurred in the absence of some other coinfection. By univariate analysis, a number of variables were shown to be significantly associated with bacterial infections; all these are shown in Table 6. No combination of variables was significant in logistic regression analysis.

DISCUSSION

Mycobacteria, fungi, and bacteria can cause localized or systemic disease. A number of immune parameters have been suggested as being potentially important for the occurrence, presentation, and outcome of these diseases, including various cytokines and cytokine profiles, T-cell antigen receptor $\gamma\delta^+$ cells, cytotoxic T cells, NK cells, CD1c expression on antigen-presenting cells, and CD14 expression on monocytes-macrophages. We had a unique opportunity to assess the relative importance of these parameters in febrile hospitalized patients with and without confirmed BSI. Most of these patients were also infected with HIV. Type I BSI are extremely unusual in non-HIV-infected persons, precluding our examination of these infections in a larger group of non-HIV-infected persons. Although our numbers are not large and extrapolation of these results beyond HIV-infected persons should be done with caution, this study represents by far the largest group of patients with type I BSI for which cytokine and immune cellular evaluation has been done.

As would be expected, HIV status was associated with clinical outcome and culture positivity, although the first association did not reach statistical significance. Only a few cell types, cytokines, and surface molecules were related to HIV positiv-

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ity, mortality within the HIV-positive population, or organism isolation. Most striking were two discrete clusters of association: (i) the percentage of $CD3^+$ $CD16/56^+$ cells producing TNF-α associated with LNE, HIV positivity, and mortality and (ii) the percentage of $CD3^+$ $CD8^+$ cells making IL-8 or IFN-γ and the percentage of monocytes expressing CD14 associated with the organism isolated from the blood. Of note was the lack of an association in either cluster with lymphocyte counts or percentages. Also of note is that the cells and parameters of importance in both clusters were not those hypothesized as being associated with advancing HIV disease, i.e., a shift from type I to type II cytokines in $CD4^+$ cells, herein examined as those being $CD3^+$ $CD8^-$.

In the first cluster of associations, the risk of death increased with HIV positivity, LNE, and a decreasing percentage of $CD3^+$ $CD16/56^+$ lymphocytes producing TNF-α. This set of associations suggests that mortality was due to a potentiation of the HIV disease process but not to a type I to type II shift in $CD4^+$ cells. This potentiation may have been related in some way to the BSI, (e.g., to the relative virulence of the BSI) but was not related to the type of BSI per se. Further, one is lead to conjecture as to whether, in the enlarged lymph nodes as in the blood, these cells were involved in the immune system's attempt to contain the HIV infection. If so, the one deceased participant who did not have LNE might have represented a case of lymph node exhaustion or atrophy.

NK cells were once thought to play a central role in determining the initial T-cell cytokine response pattern to infection (7, 28). In our study population, they were significantly related to HIV infection but not to BSI. More striking, we found a significant inverse association between TNF-α production by $CD3^+$ $CD16/56^+$ cells, LNE, and mortality. Most NK cells do not express CD3, although CD3 expression has been occasionally reported in the context of tumors or HIV infection (29). Also, our permeabilization procedure would lead to staining of cytoplasmic, as well as surface, CD3; cytoplasmic CD3 in surface $CD3^-$ NK cells has not been examined. Our data suggest that surface and/or cytoplasmic $CD3^+$ cells positive for CD16 and/or CD56 may represent an important and currently unexamined cell population in regard to HIV infection. Further, the inverse association we found between TNF-α production by these cells, LNE, and death may be highly pertinent to the current controversy over the relative benefits and harm caused by TNF-α in the context of HIV and OI (8, 9, 14, 19, 22, 23, 30). Our data indicate that TNF-α production by $CD3^+$ $CD16/56^+$ may be associated with a more positive clinical outcome, especially for patients with LNE. Thus, use of medications known to affect TNF-α production, e.g., thalidimide or drugs specifically intended to inhibit TNF-α production, might lead to adverse outcomes in at least some persons with HIV infection.

The second cluster of immune findings, associated with blood culture positivity and with the particular organism isolated, involved monocytes and $CD8^+$ lymphocytes (often referred to as cytotoxic T cells, although $CD4^+$ cells can also exhibit cytotoxic activity). Specifically, these significant parameters included (i) the percentage of monocytes expressing CD14, (ii) the percentage of $CD8^+$ lymphocytes producing IL-8, and (iii) the percentage of $CD8^+$ lymphocytes producing IFN-γ. Each was included in the immune evaluation because previous studies have suggested they might be related to various organisms causing BSI. CD14 is a monocyte-macrophage surface molecule that can interact with both gram-negative bacterial lipopolysaccharide and with mycobacterial lipoarabinomannan (13, 25). IL-8 is a cytokine frequently studied in relation to bacterial sepsis but only infrequently evaluated in type I infection (16, 22). IFN-γ is a cytokine disproportionately produced by $CD8^+$ cells compared to that produced by $CD4^+$ cells, even in healthy humans, (11) and is felt by some to play an important role in HIV infection (3, 4, 12), toxoplasmosis (27), and murine *M. tuberculosis* (22). We found these three parameters varied in relation to the presence or absence of a positive blood culture and also in relation to the type of organism isolated from the blood. If these results, based on a single group of patients, can be replicated in other groups, they may prove useful in predicting the likelihood of a blood culture becoming positive and even what type of organism will be isolated from the blood. This could assist in decisions concerning presumptive therapy for HIV-related OI that usually require prolonged culture, such as mycobacteria.

In summary, many cytokines and immune cell types have been suggested as being important determinants of disease and outcome in OI. We wished to directly examine the immune cells responding to an infection. Thus, we evaluated peripheral blood immune cells of patients with BSI rather than those of patients with localized infections. In this study population, we found that only a few immune cell types and only three of their related cytokines were significantly associated with disease outcome and the infecting organisms. These findings were not suggestive of a type I to type II cytokine shift in advancing HIV disease but, rather, supported the importance of $CD16/CD56^+$ and $CD8^+$ cells in HIV and type I infections.

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