

Intracellular cytokines may model immunoregulation of abacavir hypersensitivity in HIV-infected subjects

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Background: The clinical treatment of patients with HIV and adverse drug events may be enhanced by an understanding of the underlying mechanisms. About 4% of patients with HIV receiving the potent antiretroviral drug abacavir develop a hypersensitivity reaction. This idiosyncratic reaction appears to have an immunologic component that has yet to be defined. Given that the T-cell type 2 cytokine IL-4 may be overproduced by patients with allergy or other immunologic dysregulation, an index cytokine profile could help elucidate the character of a drug-specific hypersensitivity reaction.

Objective: Quantitation of the production of the type 2 IL-4 and the counterregulatory type 1 cytokine IFN- γ in patients with abacavir-related hypersensitivity.

Methods: Intracellular cytokines were enumerated in blood T cells by flow cytometry. Subjects were grouped for evaluation as patients with a hypersensitive response after abacavir treatment, patients initiating abacavir who also were evaluated again after 1 month on abacavir, patients on abacavir for 6 months without hypersensitivity, and HIV-naïve control individuals.

Results: There was a significant association between increased IL-4 production by CD4 and CD8 T lymphocytes and hypersensitivity reactions to abacavir. Lymphocytes from hypersensitive subjects expressed CD28 and the anti-HIV chemokine macrophage inflammatory protein 1 β with a frequency comparable with HIV-naïve control cells,

suggesting the possibility that the activated T cells from patients with hypersensitivity are functional.

Conclusion: The expansion of type 0 and type 2 T cells phenotyped by IL-4 production may correlate with abacavir-associated hypersensitivity. The data suggest a cytokine bias that may facilitate B-cell differentiation and downregulate T-cell cytotoxic responses. (*J Allergy Clin Immunol* 2005;115:1081-7.)

Key words: Hypersensitivity, HIV, adverse drug reactions, cytokines, IFN- γ , IL-4, flow cytometry, antidrug antibodies

Abacavir (an active ingredient in Ziagen and Trizivir [both GlaxoSmithKline, Research Triangle Park, NC]) is a potent nucleoside analogue reverse transcriptase inhibitor that has been associated with hypersensitivity reactions in approximately 4% of patients receiving the drug.^{1,2} The reaction is well characterized. Symptoms include fever, skin rash, gastrointestinal symptoms (abdominal pain, nausea, diarrhea), generalized malaise, and respiratory symptoms (cough, shortness of breath) and first present at a median of ~11 days. Rapid recurrence of hypersensitivity symptoms has been reported after rechallenge with abacavir.^{3,4}

Idiosyncratic reactions to abacavir appear to have an immunological basis, probably coupled with a metabolic component, yet the eliciting immunogen remains elusive. The clinical manifestations of abacavir-associated hypersensitivity reaction suggest a delayed immune response with cellular involvement rather than an immediate hypersensitivity reaction. Recent studies have demonstrated the potential complexity of cellular responses to an antigenic challenge, such as those seen to gelatin in vaccine preparations,⁵ eggs,⁶ nickel,⁷ amoxicillin,⁸ and the fungus *Trichophyton*.⁹ Analysis of infiltrating cells in delayed-type hypersensitivity skin test reactions has shown the presence of both type 1 and type 2 antigen-specific T cells.⁵⁻⁹ In addition, antigen-specific T cells that preferentially produce the type 2 cytokine IL-5 rather than the type 1 cytokine IFN- γ have been noted.⁸ Profiling of cytokines that may regulate such an immune response may provide data regarding immunologic function. The use of flow cytometry has allowed subtle changes in

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Abbreviations used

ABC: Abacavir
 FBS: Fetal bovine serum
 HAART: Highly active antiretroviral therapy
 HSR: Hypersensitivity
 MIP-1 β : Macrophage inflammatory protein 1 β
 PMA: Phorbol 12-myristate 13-acetate

cytokine production within T-cell subsets to be defined by simultaneous staining for lymphocyte phenotype coupled with intracellular cytokine staining.

The current study was conducted to determine the frequency of IL-4-producing and IFN- γ -producing T lymphocytes in patients with hypersensitivity to abacavir. These 2 index cytokines are implicated in promoting or suppressing antibody-mediated immune responses, respectively.^{10,11} Our objective was to determine whether imbalances in the production of these cytokines were associated with hypersensitivity to abacavir, and to establish whether the underlying mechanisms were those of a classic delayed-type hypersensitivity response involving an expansion of IFN- γ ⁺ type 1 T cells. Our data show that the delayed hypersensitivity reaction to abacavir is not associated with increased IFN- γ production after mitogen stimulation. Instead, we demonstrate a diverse cytokine response in abacavir-associated delayed hypersensitivity reactions that involves expansion of IL-4⁺ as well as IL-4⁺IFN- γ ⁺ (double-positive) CD4 and CD8 T cells.

METHODS

Clinical material

Four groups of individuals were studied for cytokine profiles and antibody to abacavir. These included (1) HIV-naïve controls (blood donors from the South Thames Blood Transfusion Service; HIV_Naive); (2) HIV-positive individuals with progressive disease who had received previous antiretroviral treatment and were enrolled prospectively and were studied before abacavir treatment (ABC_Initiators) and again 1 month after initiation of highly active antiretroviral therapy (HAART) + abacavir without hypersensitivity (ABC_1Mo_NoHSR); (3) HIV-positive individuals (unique from group 2) who received HAART + abacavir for at least 6 months without hypersensitivity (ABC_6Mo_NoHSR); and (4) HIV-positive individuals who received HAART + abacavir and were recently diagnosed with hypersensitivity (HSR). Subjects were enrolled in the study, which had been approved by the hospitals' Institutional Review Board, on obtaining signed consent. A diagnosis of hypersensitivity was made by the treating physician on the basis of clinical observations of fever, and malaise and/or rash compatible with drug hypersensitivity; blood samples were obtained at a median of 3 days after diagnosis of hypersensitivity. Table 1 summarizes the CD4 cell count and HIV-1 viral load at evaluation. Recruitment procedures and the study population have been described previously.¹² All patients had relatively advanced disease (median CD4 cell counts $<350 \times 10^6/L$) and were generally initiating abacavir because they had failed to respond to a previous antiretroviral combination.

Cell and plasma sample preparation

Heparinized blood was centrifuged briefly, and the plasma was removed and stored at -20°C for later antibody analysis. Resuspended PBMCs were isolated by density gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway) within 24 hours of venipuncture. PBMCs from 2 subjects (ABC_Initiator and HSR) were assayed fresh in preliminary experiments for comparison with cells preserved at -70°C in fetal bovine serum (FBS; Sigma/Aldrich, Poole, United Kingdom) + 10% dimethylsulfoxide (Sigma/Aldrich) in aliquots of 5×10^6 to 20×10^6 cells per vial. The preliminary experiments demonstrated that the percentage of CD4 and CD8 cells was within 7% of fresh PBMCs for study blood samples. There were only small differences for intracellular cytokine percentages, with IFN- γ frequencies somewhat lower and IL-4 frequencies somewhat higher from frozen cells. All blood samples from all subject groups were handled identically.

Frozen PBMCs were thawed and resuspended in RPMI + 10% FBS with 40 IU/mL IL-2 (National Institute for Biological Standards and Control, United Kingdom) in 24-well plates at $1 \times 10^6/mL$ and cultured in the presence of 50 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma/Aldrich) and 400 ng/mL ionomycin (Sigma/Aldrich). Preliminary experiments compared stimulation of cells for 4, 16, or 20 hours at 37°C ; 20-hour cultures produced optimal cytokine-positive cell percentages and so were routinely used. The protein transport inhibitor brefeldin A (Sigma/Aldrich) was added at 5 $\mu\text{g}/\text{mL}$ for the last 16 hours of culture. Stimulated cells were washed once and permeabilized with Orthopermeafix (Ortho-Clinical Diagnostics, Amersham, Berks, United Kingdom).

Immunofluorescent staining and analysis

Fixed and permeabilized PBMCs were plated at 2×10^5 per well in 96-well U-bottom Flexi-plates (Falcon; BD/Pharmingen) and stained in a single step with the following antibodies: fluorescein-conjugated anti-IFN- γ , phycoerythrin-conjugated anti-IL-4, 5-N-N'-diethyl-tetramethylindodicarbocyanine-conjugated anti-CD8, and allophycocyanin-conjugated anti-CD3 (all from BD/Pharmingen, Cowley, Oxford, United Kingdom) for 40 minutes at 4°C . Additional markers studied were IL-2, CD28, and macrophage inflammatory protein 1 β (MIP-1 β). Controls included samples stained for each single antibody and isotype-matched fluorochrome-labeled control antibodies as well as samples stained with 3 antibodies and 1 isotype control. Stained samples were washed 3 times in cold PBS + 2% BSA and then resuspended in PBS + 2% BSA + 1% FBS.

Each analysis was conducted on 10,000 to 50,000 events acquired by using a FACSCalibur (BD/Pharmingen) flow cytometer, and listmode data files were analyzed by using CellQuest software (BD/Pharmingen). The lymphocyte gate was defined manually on forward-scatter and side-scatter characteristics for each sample. CD4 cells were defined as the CD3⁺CD8[−] subset within the live lymphocyte gate. Analysis of cytokine production was performed on CD3⁺CD8⁺ and CD3⁺CD8[−] subsets. Results were expressed as the percentage of a subset that stained positive for each cytokine.

Detection and characterization of antibodies to abacavir by fluoroimmunoassay

Abacavir conjugated to keyhole limpet hemocyanin was used to produce rabbit antiabacavir standard antibody, as previously described,¹³ and as a plate antigen to detect human antibody to abacavir. Diluted plasma was incubated on abacavir-KLH-coated 96-well plates, and binding was detected with biotin-conjugated mouse antihuman IgG-biotin (#B3773; Sigma/Aldrich, St Louis, Mo), goat antihuman IgM-biotin (#B1265; Sigma/Aldrich), or mouse antihuman IgE-biotin (#9160-08; Southern Biotechnology Associates,

TABLE I. Subject groups and disease status information

Patient group	Description	n	Median CD4 count* × 10 ⁶ /L (range)	Viral load, median (range) copies/mL
HIV_Naive Controls	HIV-naive controls; anti-HIV antibody- negative	15	—	—
ABC_Initiators	Abacavir-naive, initiating new therapeutic regimen with HAART + abacavir; treatment- experienced	20	321 (71-697)	4,049 (<50 to 5 × 10 ⁵)
ABC_1Mo_NoHSR	ABC_Initiators evaluated after 1 mo HAART + abacavir; treatment- experienced	20	345 (55-702)	60 (<50-4334)
ABC_6Mo_NoHSR	HAART + abacavir for 6 mo; treatment-experienced; no hypersensitivity	32	330 (117-999)	<50† (<50-84,275)
HSR	HAART + abacavir for median 12 d (treatment stopped on hypersensitivity symptoms); treatment-experienced	27	250 (11-1061)	340‡ (<50 to 5 × 10 ⁶)

*CD4 counts not statistically different among HIV-positive groups.

†ABC_6Mo_NoHSR compared with ABC_Initiators, $P < .0001$ (significant at $P \leq .0025$ adjusted for multiple comparisons across all combinations).‡HSR compared with ABC_6Mo_NoHSR subjects, $P < .0001$ (significant at $P \leq .0025$ adjusted for multiple comparisons across all combinations).

Birmingham, Ala), followed by europium-labeled streptavidin (Wallac/Perkin-Elmer; Gaithersburg, Md) and counted by time-resolved fluorescence on a Delfia plate reader (Wallac/Perkin-Elmer). The signal was corrected for nonspecific binding of normal human serum and for binding to KLH alone. Antibody titers greater than 30 were considered significant.

Statistical analysis

A Wilcoxon rank-sum test was used to compare median frequencies of type 0, type 1, and type 2 lymphocyte subsets between the HSR group and the ABC_1Mo (no HSR) group. Multiple comparisons have been performed, and we therefore decided to use a Bonferroni adjustment; each test was performed at the .0083 level of significance. We also examined the relationship between CD4 and CD8 cell cytokine (IL-4 and IFN- γ) production in patients with HSR compared with ABC_1Mo (no HSR) patients by using a 2-sample 2-tailed t test and a Bonferroni-adjusted significance level of $P \leq .0125$. Analysis of contingency tables for antibody frequency per group was performed by using JMP version 5.1.1 software (SAS Institute, Cary, NC). Differences in the number of subjects in each cohort make statistical comparisons among the larger cohorts more powerful than comparisons among the smaller cohorts.

RESULTS

Suitability of culture conditions for intracellular cytokine production in HIV-infected PBMCs

Stimulation of PBMCs with PMA and ionomycin was most effective in a 20-hour culture, with brefeldin A added after the first 4 hours. There was no difference in the frequency of cytokine-producing cells between stimulated

cultured fresh and frozen PBMCs. However, there was a moderate increase in the amount of debris (low forward scatter) from frozen cells (data not shown).

Effect of HAART + abacavir on CD4 counts and viral loads

CD4 counts and HIV-1 RNA of the HIV⁺ groups are summarized in Table I. There was a trend toward a lower CD4 cell count among the HSR cases. Among subjects with progressive disease before initiation of a new therapeutic regimen of HAART + abacavir (ABC_Initiators), viral load dropped from a median of 4049 copies/mL to 60 and <50 copies/mL, 1 month and 6 months, respectively, after initiation of HAART. Viral load of the HSR group at a median of 12 days after treatment initiation was a median of 340 copies/mL.

IL-4 production is impaired in abacavir-naive initiators compared with HIV-naive controls and is reversed to normal after 6 months on HAART + abacavir

The frequency of lymphocytes that express intracellular IL-4 is shown in Fig 1. IL-4 production in CD4 and CD8 T cells in HIV⁺ subjects with progressive disease who had failed to respond to previous therapy and who just initiated HAART + abacavir (ABC_Initiators) was significantly lower than that of HIV-naive controls. This impairment was normalized for both CD4 and CD8 cells in subjects who had received 6 months of HAART + abacavir (ABC_6Mo_NoHSR). Unlike IL-4 production, the frequency of IFN- γ ⁺ CD4 and CD8 positive T cells

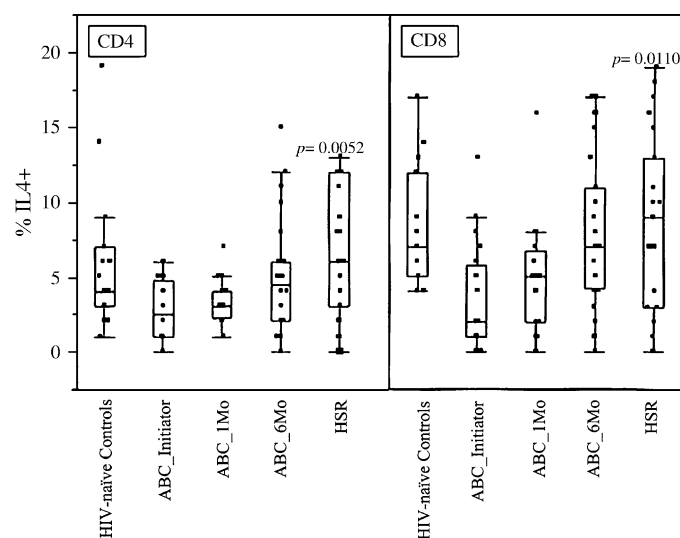


FIG 1. Intracellular IL-4 production in CD3⁺CD4⁺ and CD3⁺CD8⁺ lymphocytes. The *line across each bar* represents the median, and the *lower and upper ends of the box* are the 25th and 75th quartiles; probable data outliers are shown beyond the *whiskers*. HSR subject responses were compared against ABC_1Mo (ABC_Initiators evaluated 1 month after initiation of HAART + abacavir) by *t* test, and IL-4 production was found to be significantly higher in both CD4 and CD8 cell populations (*P* values are shown).

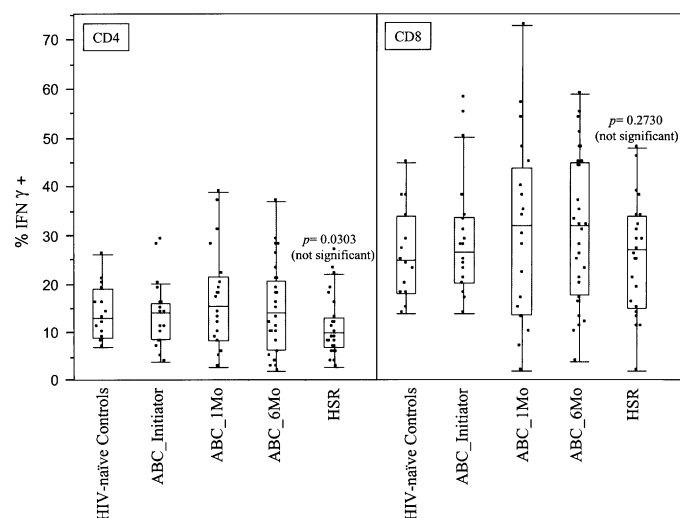


FIG 2. Intracellular IFN- γ production in CD3⁺CD4⁺ and CD3⁺CD8⁺ lymphocytes. The *line across each bar* represents the median, and the *lower and upper ends of the box* are the 25th and 75th quartiles; probable data outliers are shown beyond the *whiskers*. HSR subject responses were compared against ABC_1Mo (ABC_Initiators evaluated 1 month after initiation of HAART + abacavir) by *t* test, and IFN- γ production was found to be lower in both CD4 and CD8 cell populations, but not statistically significant after adjustment for multiple comparisons (*P* values are shown).

remained unaltered in HIV⁺ subjects compared with HIV-naïve controls (Fig 2).

IL-4 production potential is elevated in the hypersensitive subject group compared with subjects at initiation of HAART + abacavir and after 1 month of therapy

The median frequency of IL-4⁺ CD4 and CD8 T cells was significantly higher in HIV⁺ subjects with an HSR

reaction potentially related to abacavir compared with subjects both before initiation of HAART + abacavir and after 1 month of treatment (Fig 1). In the CD4 compartment, there was a 2-fold expansion of IL-4⁺ T-cell numbers compared with those patients at initiation and after 1 month. In the CD8 compartment, the HSR group had almost 5-fold higher median percentages of IL-4⁺ T cells compared with ABC_Initiators and 2-fold higher numbers of IL-4⁺ T cells in those patients after 1 month (ABC_1Mo_NoHSR). In both compartments, the

TABLE II. Cross-sectional median frequencies of type 0, type 1, and type 2 lymphocyte subsets as defined by intracellular cytokine phenotype of stimulated lymphocytes*

Subset	HIV_Naive Controls (n = 15)	ABC_Initiators (n = 20)	ABC_1Mo_NoHSR† (n = 20)	ABC_6Mo_NoHSR (n = 32)	HSR (n = 27)
T _H 0 (IFN- γ ⁺ IL-4 ⁺)	2.0	1.0	1.0	1.0	2.0
T _H 1 (IFN- γ ⁺)	10.0	13.0	13.5	12.5	8.0
T _H 2 (IL-4 ⁺)	2.0	1.5	2.0	2.5	4.0
T _C 0 (IFN- γ ⁺ IL-4 ⁺)	5.0	1.0	2.0	5.0	5.0
T _C 1 (IFN- γ ⁺)	21.0	26.5	29.0	25.5	21.0
T _C 2 (IL-4 ⁺)	2.0	1.0	2.0	3.0	3.0
					<i>P</i> = .0004‡

*The statistical comparison of the HSR group with ABC_1Mo used *P* values adjusted for multiple comparisons and was significant for Tc2 cells. All cell types defined by IL-4 secretion showed increased IL-4 production in the HSR group compared with ABC_1Mo.

†Subjects were ABC_Initiators who were evaluated again after 1 month on HAART + abacavir.

‡Statistical significance when compared with ABC_1Mo_noHSR after adjustment for multiple comparisons.

frequency of IL-4⁺ T cells in the HSR group was higher than in subjects in the ABC_6Mo_NoHSR group but did not reach statistical significance.

Type 2 and type 0 T cells are expanded in the hypersensitive subject group

The HSR subject group had an increased median frequency of T_H0 and T_H2 IL-4-producing CD4 T cells compared with ABC_Initiators (Table II; Fig 1). Conversely, the frequency of T_H1 IFN- γ -producing CD4 T cells was lower in the hypersensitive group compared with ABC_Initiators (Table II; Fig 2).

A similar overall profile was observed for CD8 T cells (Table II), which showed an expansion of T_C0 and T_C2 IL-4-producing cells in the HSR group compared with ABC_Initiators, and a reduction in the median frequency of T_C1 IFN- γ -producing CD8 cells. There was also a decreased frequency of T_H1 and T_C1 cells in HSR subjects compared with ABC_Initiators.

Expansion of IL2⁺ CD4 T cells in hypersensitive subjects compared with patients on HAART + abacavir therapy for 6 months without hypersensitivity

The median frequency of IL2⁺ CD4 and CD8 T cells was lower in ABC_Initiators than HIV-naive controls (Table III), but this trend was not statistically significant, and it was even less notable in the ABC_6Mo_NoHSR subjects compared with the HIV-naive controls. However, patients with hypersensitivity had a similar percentage of IL2⁺ CD4 and CD8 T cells that was higher than both ABC_Initiators and ABC_6Mo_NoHSR subjects (Table III). Thus, the hypersensitivity response appeared to be associated with expansion of IL2⁺ CD4 T cells, suggestive of heightened immune responsiveness, which ex-

TABLE III. Frequency of IL2⁺, CD28⁺, and MIP-1 β ⁺ CD4 and CD8 T cells in HIV-naive controls, subjects initiating abacavir, hypersensitive subjects on abacavir, and subjects on HAART + abacavir therapy for 6 months without hypersensitivity*

	HIV_Naive Controls	ABC_Initiators	ABC_6Mo_NoHSR	HSR
IL2 ⁺	14.0	6.0	8.4	12.1
CD4	(5.2-22.0)	(2.1-30.0)	(3.2-16.0)	(5.1-30.0)
IL2 ⁺	7.0	2.0	3.1	5.9
CD8	(2.0-11.0)	(0.9-20.0)	(1.0-9.0)	(0.8-9.0)
CD28 ⁺	61.1	58.4	50.5	60.8
CD4	(43.5-75.9)	(22.1-74.4)	(23.9-64.5)	(37.7-71.0)
CD28 ⁺	42.9	42.0	26.5	27.5
CD8	(28.8-52.9)	(32.2-62.7)	(15.6-54.7)	(11.1-44.1)
MIP-1 β ⁺	2.9	1.7	3.2	3.6
CD4	(0.1-5.4)	(0.4-8.9)	(0.4-42.9)	(0.1-10.5)
MIP-1 β ⁺	11.3	3.6	9.1	9.4
CD8	(0.9-17.2)	(0.8-8.4)	(4.7-32.5)	(1.0-28.5)

*Data are median frequencies; the range is in parentheses. None reached statistical significance after adjustment of *P* values for multiple comparisons; however, the HSR group trended toward higher IL-2 and MIP-1 β production in both CD4 and CD8 cells compared with ABC_Initiators.

ceeded that observed in patients without hypersensitivity after 6 months of antiviral treatment.

Additional markers indicative of immunologic responsiveness were also evaluated. CD28, a marker of T-cell activation and proliferation,¹⁴ and MIP-1 β , a key anti-HIV chemokine,^{15,16} were evaluated for expression in CD4 and CD8 lymphocytes. Table III shows the median and range of CD28⁺ and MIP-1 β ⁺ CD4 and CD8 T cells; no significant differences were noted in the percentage of CD28 or MIP-1 β ⁺ T cells between the various groups.

Binding antibody specific to abacavir is detectable in plasma from hypersensitive subjects as well as HIV-naïve controls and subjects on abacavir without hypersensitivity

The median frequency of IgG antibody to abacavir among the various subject groups was 19.5% and was not statistically different for the hypersensitive subjects compared with any other group by either the likelihood ratio test ($P = .7629$) or the Pearson χ^2 test ($P = .7652$). One ABC_Initiator had low-titer IgM antibody; 1 subject in the ABC_6Mo cohort had IgE (ABC_6Mo) antibody. Neither subject developed hypersensitivity.

DISCUSSION

This study demonstrates that a hypersensitivity reaction to abacavir is associated with a shift in the cytokine balance toward expansion of type 0 and type 2 (IL-4⁺) CD4 and CD8 T cells. Two aspects of altered IL-4 production in HIV infection have been highlighted by our data. First, HIV infection impairs IL-4 production, and this decreased production potential of CD4 and CD8 T cells is evident in HIV⁺ subjects failing to respond to therapy (ABC_Initiators). Impaired production of mitogen-induced IL-4 production previously noted in HIV infection^{17,18} might reflect the loss of T_H2 or T_H0 cells caused by preferential virus replication in these cells^{19,20} and/or IFN- γ -induced IL-4 downregulation. The latter would be particularly applicable in HIV⁺ subjects in whom an expansion of IFN- γ ⁺ T cells has been noted.^{17,18} The effect of this loss of CD4 IL-4 production on the immune potential would depend to an extent on the compensatory effect provided by the CD8 compartment, which is well recognized to be expanded in patients with HIV as they progress to disease.

Second, IL-4 production apparently recovers by 1 to 6 months of antiretroviral treatment with HAART + abacavir, with IL-4 levels comparable with those in HIV-naïve controls. By comparison, the expansion of IL-4⁺ T cells in HSR subjects occurred within the median time of 12 days, with levels similar to the HIV-naïve controls. Therefore, the IL-4 response in subjects with a hypersensitivity reaction is probably a consequence of the hypersensitivity reaction at the time of sampling (median 3 days after onset of HSR) rather than a response to HAART + abacavir treatment, although precisely time-matched treated controls (ie, 3 days on HAART + abacavir) were not available. It is also possible that CD4 IL-4 responses were preserved in subjects who later developed hypersensitivity.

The expansion of IL-4⁺ T cells in hypersensitive subjects may represent an expansion of specific memory T cells responding to antigenic stimulation. This response could be initiated if abacavir or a metabolite acted as a hapten (ie, the hapten hypothesis for the mechanism of idiosyncratic drug reactions,²¹ whereby drugs or reactive metabolites of drugs act as haptens and irreversibly bind to

proteins, resulting in an immune response to the altered protein) and triggered abacavir-specific immunologic reactivity. The demonstration of an expansion of abacavir-specific type 2 and type 0 T cells in hypersensitive subjects also provides immune response data that might be linked to the recent observation of a strong association between MHC alleles and abacavir hypersensitivity.^{22,23} Increased IL-4 production in hypersensitive subjects may also reflect a lower threshold for activation of T cells irrespective of cell specificity. This alternative to the hapten hypothesis proposes that a drug may act as a superantigen by noncovalent interaction with a major histocompatibility complex molecule, resulting in a more general immune response.²⁴ However, the mechanism for preferential upregulation of type 0 and type 2 cells remains unknown. Future work on the specificity of type 2, type 1, and type 0 T cells in hypersensitive individuals should help clarify the underlying mechanisms. In addition, a study design with improved statistical power (especially larger group sizes that are equivalent between test population and control group) would enhance the ability to discern statistically significant differences in cytokine profiles.

Although there has been some suggestion of an increased propensity of HIV patients to atopy and hypersensitivity, and there have been reports of a type 2 rather than a type 1 profile in patients with HIV as they progress to disease,²⁵ more recent data suggest that patients with HIV have a type 1 cytokine profile irrespective of disease stage with preservation of IFN- γ production, especially by CD8 T cells, even in late-stage disease.²⁶ Therefore, it is unlikely that the increased IL-4 production to abacavir is a result of patients with HIV having an intrinsic susceptibility to hypersensitivity simply because they produce high IL-4.

Among the immunoregulatory activities of IL-4 is B-cell differentiation and antibody class switching to IgE. In addition, type 2 cytokines (eg, IL-5) may participate in the production of eosinophils. Thus, a search for specific immunoglobulin was warranted despite the absence of clinical signs of antibody-mediated adverse events or eosinophilia. The data reveal no clear association of antiabacavir binding activity with hypersensitivity, although a relatively high frequency of binding activity to abacavir was shown. It is interesting that pre-existing antibody that can cross-react with abacavir is present in individuals who have never received abacavir, and also that antiabacavir IgG titers rise slightly in hypersensitive patients. However, statistically significant differences among means for this low-titer antibody are likely to be biologically irrelevant. The clinical presentation of hypersensitivity associated with abacavir does not suggest the involvement of IgE antibody, and IgE was detected at low titer in only a single individual.

The expansion of IL-4⁺ cells in hypersensitive individuals was associated with an expansion of IL2⁺ T cells, indicative of CD4 T-cell activation. Furthermore, CD28 expression and MIP-1 β production by CD4 and CD8 T cells in hypersensitive subjects was similar to that of

HIV-naïve controls, suggesting that the proliferative and antiviral potential of IL-4⁺ T cells in hypersensitive subjects is likely to be intact. Other studies in HIV-infected patients have shown that an allergic response that results in T-cell IL-4 production can also lead to T-cell IL-2 production, enabling the central role of T-cell help in immune responses during a hypersensitivity reaction.²⁷ Indeed, the expansion of T-cell IL-2 production, along with the expansion of not just type 2 but also type 0 cells (IFN- γ ⁺ and IL-4⁺ producers), the lack of a hypersensitivity-associated antiabacavir IgE antibody response, and the delay in the development of symptoms collectively indicate a type III/IV hypersensitivity reaction to abacavir rather than a classic type I hypersensitivity reaction. Although the expansion of type 2 T cells is classically associated with a type 1 hypersensitivity reaction, our studies—consistent with others²⁷—would advocate a more complex cytokine pattern associated with some type III/IV nonclassical reactions. Further work using multiplexed cytokine assays in abacavir-specific T cells should help resolve whether the induction of IL-4 in hypersensitive subjects points to an ensuing role for IL-4⁺ T cells in the clinical pathology or is merely a counter-regulatory protective immune response to viral infection.

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