IMMUNOLOGY AND CANCER

Abnormal immunological response to *Mycobacterium tuberculosis* antigens in a patient with chronic myelocytic leukemia and active tuberculosis

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ABSTRACT. The pathogenic mechanisms of immunosuppression leading to susceptibility of *Mycobacteriun tuberculosis* (MT) infection in chronic myelocytic leukemia (CML) are not clear. To address this issue, we measured the proliferative response, variation of T cell subpopuplations (CD4+, CD8+, TCR-Vδ2 and TCR-Vß8 T cells) and the cytokine profile (IL-1β , IL-2, IL-4, IL-6, IL-10, TNF-α ,IFN-γ) after MT stimulation of peripheral blood mononuclear cells (PBMC) in a patient with concomitant CML and active pulmonary tuberculosis. The results were compared to four patients with active pulmonary tuberculosis and no other coexistent diseases. The immunologic response to phytohemagglutinin (PHA) was also evaluated. In contrast to controls, the CML PBMC failed to proliferate in response to MT antigens. *Mycobacterium*-reactive CD4+, Vδ 2 and Vß8 T cells did not expand after MT stimulation of the CML PBMC. In MT antigens-stimulated cultures from the CML patient, IL-2 was not produced and mild reduction of IL-1ß and INF- γ were observed. In contrast, IL-10 was markedly elevated in these cultures. Similarly, PHA-stimulated PBMC from the CML patient showed no expansion of CD4+ and CD8+ T cells. In these cell cultures, INF- γ concentration in supernatants was decreased and IL-10 was significantly elevated. This study suggests that patients with CML may present a profound immunosuppression of essential cellular and molecular immune effectors, a scenario which might contribute to the development of active tuberculosis. These findings further support the need of establishing immunotherapeutic modalities with potential value for myeloproliferative disorders. *Keywords: T cells, Cytokines, Mycobacterium tuberculosis, Chronic myelocytic leukemia*

The incidence of mycobacterial infections is higher among patients with cancer (1-3). Those with lung cancer and lymphoproliferative disorders, especially leukemia, are the most susceptible. The disease process and the anti-tumor treatment contribute to the immunosuppression resulting in a higher prevalence of tuberculosis among these patients. Most frequently, tuberculosis results from a reactivation of an earlier infection. The mechanisms of immunosuppression resulting from disease are unclear for patients with leukemia, specifically those with chronic myelocytic leukemia (CML).

Cellular immunity plays a central role in the immune response against *Mycobacterium tuberculosis (*MT*)*. Antigen-stimulated αβ CD4+ T cells activate macrophages at the site of infection to limit the proliferation and spread of MT (4). Also, γδ T cells with the Vγ9/Vδ2 T cell receptor proliferate in response to mycobacterial antigens and accumulate in MT infectious lesions (5-6). A broad spectrum of cytokines, including interleukin 2 (IL-2), interleukin 10 (IL-10), interferon gama (INFγ) and tumor necrosis factor alpha (TNF-α) are secreted by both T cell subsets in response to MT stimulation (7). INF-γ and TNF-α are important cytokines in conferring resistance to *Mycobacterium tuberculosis*

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since they induce tuberculostatic macrophage function (8). In addition, adoptive transfer experiments and studies using knockout mice have shown that INF-γ is the essential activating cytokine and that CD4+ and CD8+ T cells are important in the protection (9-10).

Pathogenic mechanisms resulting in cellular immunity dysfunction will contribute to the development of active tuberculosis. In CML there are several studies regarding the immune response to leukemic cells, but few, on the competence of the immune system, particularly against infectious agents. One of these latter studies show that monocytes of CML patients have decreased expression of HLA-DQ and impaired antigen-presenting capability (11). Also, T cells from bone marrow of CML patients suppress autologous bone marrow mononuclear cells (12). This suppression appears to be mediated by CD4+ T cells. Following bone marrow transplantation, the immunological reconstitution, especially of T cells, is slower in patients with CML than in patients with acute myeloid leukemia or acute lymphoblastic leukemia (13). These studies suggest that CML patients might have a dysfunctional cellular immunity against infectious agents but further studies would be necessary to elucidate the precise immunopathogenesis.

To examine the immune response to MT in CML, we evaluated the proliferative response, T cell subset expansion, and cytokine secretion of peripheral blood mononuclear cells (PBMC) derived from a CML patient with active pulmonary tuberculosis and compared these findings with patients having active pulmonary tuberculosis but no coexistent diseases. A consistently abnormal immunological response in all cellular function studies was documented for the PBMC derived from the CML patient. These abnormalities might help to explain the immnunopathogenic mechanisms that render CML patients susceptible to develop active tuberculosis.

Materials and Methods

Subjects. The study population consisted of five patients with active pulmonary tuberculosis. All patients had *Mycobacterium tuberculosis* confirmed by bacteriological studies. At the moment of blood collection for experimental studies the patients were in their second to third month of anti-tuberculosis treatment which included isoniazid, rifampin and pyrazinamide. One patient had CML and the other four patients (three males, one female) had no other systemic diseases or other debilitating conditions. None of these patients had clinical evidence of extra-pulmonary tuberculosis and all patients were HIV negative.

The patient with CML is a 39-years-old male refered to our University Hospital with active pulmonary tuberculosis. Eight months prior to the mycobacterial infection he was referred to our institution because of presumed CML. At that time, complete blood cell count showed a white blood cell count of 53,600/ mm^3, hemoglobin 12.8 g/dl and a platelet count of 998,000/ mm^3. Bone marrow biopsy revealed a hypercellular bone marrow with predominance of myeloid and magakariocytes series with shift and abnormal forms consistent with chronic myeloid leukemia. Blasts were below 5%. Chromosomal analysis confirmed a complex Philadelphia chromosome three way translocation; the breakage points were 3q23-25, 9q34 and 22q11. The patient was started in hydroxyurea and remained in this medication by the time of blood collection for experimental studies. Prior to, or during experimental studies, the patient did not evolve into a myeloblast transformation or received any other anti-tumor treatment including interferon-α.

Blood samples used for controls were obtained from 20 healthy individuals. Informed consent was signed by all subjects.

Separation of PBMC. Blood samples were collected in heparinized tubes and diluted 1:1 by volume at room temperature in PBS pH 7.4, Ca++, Mg++ free (Gibco, Grand Island, NY). The diluted sample was overlaid on Ficoll-Pacque (Pharmacia Biotech, Piscatawacy, NJ) at room temperature. Samples were centrifuged at 2,500 rpm for 20 minutes at room temperature. The PBMC layer was collected and cells were washed once with PBS. PBMC were resuspended in 10% FBS-RPMI 1640 medium.

Proliferation response of PBMC to MT antigens and PHA. PBMC (1 x 10^5 / well) were incubated for seven days in the presence or absence of 10 μg/ml of sonicated lyophilized *Mycobacterium tuberculosis* strain H37Ra (Difco, Detroit,MI), or one μg/ml phytohemagglutinin (PHA) (Sigma, St. Louis, MO). Appropriate controls were included. Assays were run in triplicates. Eighteen hours prior to the end of the incubation period, the PBMC were pulsed with one μCi/ml of [^{3}H]-thymidine (Moravek Biochemicals, Brea, CA). The cells were harvested (PHD cell harvester, model 200A, Cambridge Technology, Watertown, MA) and twenty-four hours later, scintillation fluid (Econo SX, Fisher, Fair Lawn, NJ) was added. β-emission was measured in a Beckman scintillation counter (model LS-3801). Results were expressed in mean counts per minutes (CPM) or stimulation (proliferative) index (mean cpm of stimulated PBMC - mean cpm of unstimulated PBMC/ mean cpm of unstimulated PBMC). Proliferation experiments were carried out in RPMI medium supplemented with penicillin/streptomycin,

glutamine, HEPES buffer and 10% FBS (Gibco, Grand Island, NY).

Cell cultures for T cell subpopulations and cytokine profile analysis. PBMC (1 x 10^6 / well) were incubated for seven days in the presence or absence of 10 μg/ml of sonicated lyophilized *Mycobacterium tuberculosis* strain H37Ra, or one μg/ml PHA. Appropriate controls were included. Cell cultures were carried out in RPMI medium supplemented with penicillin/streptomycin, glutamine, HEPES buffer and 10% FBS (Gibco, Grand Island, NY). After seven days of culture, cell cultures were collected and centrifuged at 1,000 rpm. Supernatant was collected for determination of cytokine concentration and PBMC were resuspended in staining media for FACS analysis.

Phenotypic analysis of peripheral blood cells and cell cultures. Peripheral blood counts of CD4+ and CD8+ T cell subsets were determined from EDTA-treated blood using either a lysed whole blood method or separated PBMC on day 1 using a method standardized to be used in FACScan brand flow cytometer (Becton-Dickinson Immunocytometry System, BDIS, San Jose, CA). Briefly, 10 ml of monoclonal combinations consisting of either anti-Leu 4- FITC/ Leu 3a - PE (CD4 + T cells) or anti-Leu 4 - FITC / Leu 2a - PE (CD8+ T cells) (Simultest Reagents, BDIS, San Jose, CA) were added to 50 μl whole blood and incubated for 15 min at room temperature, in the dark. Following a 10 min lysis step, the resulting cells were washed twice in D-PBS and fixed in O.5 % EM-grade formaldehyde.

Ficoll-hypaque separated PBMC were stained with anti-Leu 4-PE (anti-CD-3) (BDIS, CA) in combination with one of the following FITC - conjugated monoclonal antibodies: anti - TCR-Vδ2 (T Cell Diagnostics, MA), anti - TCR - Vβ8 (T Cell Diagnostics, MA) and anti - Leu-9 (anti-CD7) (BDIS, San Jose, CA). Approximately 2 x 10^5 cells were stained for 15 minutes at room temperature, in the dark, washed and fixed as stated above. Flow cytometric analysis was performed using a FACScan (BDIS, San Jose, CA) equipped with a 488 mm - argon ion laser, following set-up and calibration using the company's CaliBRITE beads and AutoCOMP software. The lymphocyte population was gated based on both light scattering and CD45 fluorescent properties. Absolute counts for the lymphocyte subsets were derived from a complete blood count, the lymphocyte fraction, and the proportion of antibody-positive cells from the cytofluorometric analysis.

Cytokine concentration in cell culture supernatants. Cell cultures were maintained at 37° C in atmosphere containing 5% CO_2 and supernatants were harvested on day seven and then stored at -70°C until measurements of cytokine concentrations were determined by using commercial ELISA kits (Gemzyme, Boston, MA) following the methods recommended by the manufacturer (14). The following cytokines were measured: IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ. Appropriate controls were included to compensate for cytokine concentrations in the media used.

Results

To assess differences in the immunological response between a patient with concomitant CML and active pulmonary tuberculosis and four patients presenting an analogous clinical course of pulmonary tuberculosis without coexistent diseases, we measured the proliferative response, changes of lymphocyte subpopulations and cytokine secretion profile of PBMC upon stimulation with MT antigens.

The first evidence showing immunological differences between the individual with coexistent CML and pulmonary tuberculosis and the other four with active pulmonary tuberculosis was the comparative yield

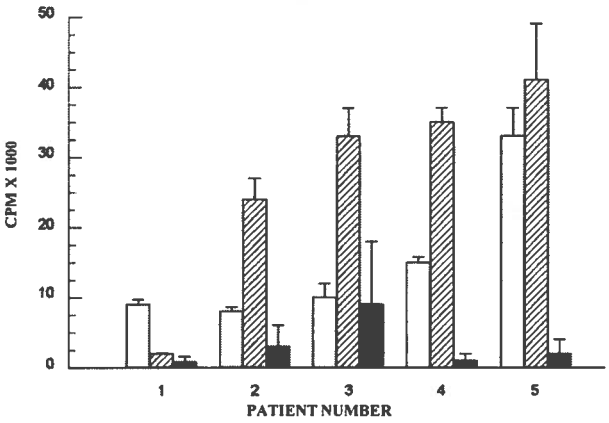

Figure 1. Proliferative response of PBMC to *Mycobaterium tuberculosis* (MT) antigen and PHA. Proliferation was measured by [^{3}H]-thymidine incorporation after 7 days of culture. Patient no. 1 had CML and active pulmonary tuberculosis; patients no. 2-5 had active pulmonary tuberculosis and no coexistent diseases. The results of unstimulated PBMC (open bars), MT antigens-stimulated PBMC (hatched bars), and PHA-stimulated PBMC (solid bars) are shown.

observed in the proliferation experiments (Fig. 1). There was an adequate proliferative response to MT antigens by the PBMC from all four patients with active pulmonary tuberculosis and no coexistent diseases (patients no. 2-5). The *Mycobacterium*-stimulated PBMC from these four patients were able to incorporate from 20 to 200% more the radioisotope-indicator of proliferative capacity than the unstimulated PBMC (Stimulation Index, SI, ranging

from 1.24 to 3.24). On the contrary, the PBMC derived from the CML patient (no.1) showed a 75% reduction in the amount of incorporated [^{3}H]-thymidine (SI=-0.24). For all patients, the proliferative response to PHA at seven days was lower than unstimulated PBMC. This finding was expected since PHA stimulation of PBMC is higher at three days of culture and declines afterwards (15).

Next, we evaluated the T cell subpopulations that have been implicated in the immune response to tuberculosis and that could be responsible for the lack of proliferative response to MT antigens by PBMC derived from the CML patient. Tables 1 and 2 show cellular counts and frequencies in fresh peripheral whole blood and in PBMC

Table 1. Total white blood cells (WBC), lymphocyte counts and T cell subpopulations in whole peripheral blood.

	PATIENT NUMBER					
	1*	2†	3†	4†	5†	Healthy Control‡
Cell type						
WBC	18500	8000	6500	9700	4400	7600±2000
Lymphocyte count	2479	1488	2190	2085	1834	2402±1430
Total T cell count	2110	1180	1490	1880	1410	1725±911
Total T cell percentage	85	79	68	90	77	73±6
CD4+ T cell count	1220	790	990	1190	900	1102±741
CD4+ T cell percentage	49	53	45	57	49	45±9
CD8+ T cell count	740	360	480	670	480	612±224
CD8+ T cell percentage	30	24	22	32	26	27±6
CD4/CD8 T cell ratio	1.6	2.2	2.0	1.8	1.9	1.7±0.6

* Patient No. 1 with CML and active pulmonary tuberculosis
† Patients No. 2-5 with active pulmonary tuberculosis only.
‡ Healthy controls mean value (n=20)

prior to stimulation with MT antigens or PHA. In whole blood, except for the expected leukocytosis observed in the CML patient, the quantification and distribution of total T cells, CD4+ T cells, and CD8+ T cells were comparable in all five patients (Table 1). They were also similar to the results obtained from 20 healthy individuals. In unstimulated PBMC, however, the percentage of total T cells, CD4+ and CD8+ T cells was lower for the CML patient (Table 2). The frequencies of V-ß8, V-δ2, and CD7- T cells were variable between the individuals and no specific tendency was observed for the CML patient.

Figure 2 shows the percentage of T cell subpopulations after seven days of culture with medium alone (unstimulated), MT antigens, and PHA. Expansion of total T cells (Fig. 2A) and CD4+T cells (Fig. 2B) from unstimulated PBMC was observed for all patients. For example, in the control unstimulated cultures, an 11-24% increase in CD4+ T cell subpopulation was observed at day 7 (Fig. 2B), as compared to day 1 (Table 2). This

Table 2. T cell subpopulation distribution in PBMC before stimulation with MT antigens and PHA.

	PATIENT NUMBER				
	1*	2†	3†	4†	5†
T Cell subpopulation (%)					
Total T cell	34	66	44	84	71
CD4+	10	40	26	52	42
CD8+	13	20	16	34	27
Beta-8	15	20	17	30	12
Delta-2	1	8	2	2	1
CD7 (-)	12	9	-	6	16

* Patient No. 1 with CML and active pulmonary tuberculosis.
† Patients No. 2-5 with active pulmonary tuberculosis only.

finding suggests the existence of pre-stimulated (conditioned or primed) T cells. When the frequency of the T cell subpopulations at day seven was compared between the unstimulated and MT-stimulated PBMC, we

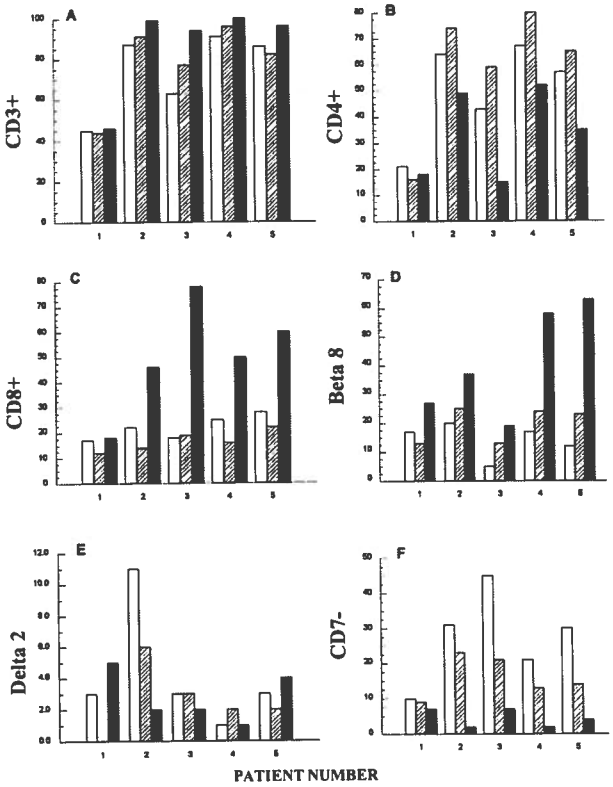

Figure 2. T-cell subpopulations after 7-days stimulation with MT antigens and PHA. The frequency of the following T cells was determined: A) Total T cells B) CD4+ T cells, C) CD8+ T cells, D) TCR-Vß8, E) TCR-Vδ2 and F) CD7- T cells . Patient no. 1 had CML and active pulmonary tuberculosis; patients no. 2-5 had active pulmonary and no coexistent diseases. The results of unstimulated PBMC (open bars), MT antigens-stimulated PBMC (hatched bars), and PHA-stimulated PBMC (solid bars) are shown.

observed further expansion (ranging from 8-17%) of CD4+ T cells for all patients except for the CML patient (Fig. 2B). The increase in CD4+ T cell percentage in cultures from the CML patient, in the presence of MT antigens, was only 6%, which was 5% lower than the yield in the corresponding control culture (11%) at day 7. Proliferation of CD4+ T cells, measured at day 7, decreased with PHA stimulation for all patients cultures.

The CD8+ T cell profile after seven days of culture is shown in figure 2C. There was no significant expansion of CD8+ T cells with MT stimulation for all patients. The frequency of CD8+ T cells was lower in the MT-stimulated PBMC than the unstimulated cells for all patients, except for patient no. 3 that was minimally increased. On the other hand, when PBMC were stimulated with PHA, a marked expansion of CD8+ T cells was observed for all patients except for the CML patient. When compared to control cultures, an increase of CD8+ T cells was observed in three of the five patients ranging from 24-32%. Interestingly, patient no. 3 showed an increase of 60%. However, no significant increase in percentage was seen for the CML PBMC.

T cells with the TCR-Vβ8 have been implicated in the immune response to MT (16). In a murine experimental model, TCR-Vβ8 cells are expanded upon mycobacterial antigen stimulation (16). In the present study, these cells were significantly elevated (12 - 30%) in fresh PBMC from all patients studied (Table 2). In two of the five patients (No. 3 and 4) this percentage was markedly reduced after culturing for seven days in the absence of antigenic or mitogenic stimuli (Fig. 2D). A further increase of 5 - 11%, as compared to day 1 (Table 1), was observed in MT antigens-exposed PBMC from all patients, except CML PBMC, in which a 4% decrease was detected. Except for cell cultures from the CML patient, T cells bearing this receptor proliferated in response to PHA.

T cells with TCR-Vδ2, also proliferate in response to mycobacterial antigens (5). Expansion of these T cells is expected in MT infection. Figure 2E shows the frequencies of Vδ2 T cells after seven days of stimulation. For all patients there was an expansion of Vδ2 T cells in the unstimulated cultures, except for patient number 4 in which a small reduction was observed. Stimulation with MT antigens resulted in further cellular expansion for patient number 4 and mild reductions for patients 2,3 and 5. In the CML patient (no. 1) cultures, the reduction was even more profound, in which no Vδ2 T cells were detected after seven days of MT stimulation. In contrast, PHA stimulation of the cells from the CML patient resulted in significant expansion from day 1 and from unstimulated cultures.

The proliferation of *Mycobacterium*-reactive γδ T cells is dependent on accessory αβ T cells with the CD4+CD45RO+CD7- phenotype (17). Most of the peripheral blood CD7- T cells carry this phenotype (18). Therefore, measurement of CD7- T cells gives a close quantification of this subpopulation of memory T cells. In the present study, a significant expansion of CD7- T cells was observed in the unstimulated PBMC at day seven for all subjects, except for the CML patient that showed a reduction (Fig 2F). The percentage increase of these cells in the control cultures at day seven ranged from 14 - 22%, when compared to day 1. These pre-stimulated cells seemed to be insensitive to further antigenic/mitogenic stimulation, as suggested by a decrease in the frequency of this T cell subpopulation in cultures stimulated by MT antigens or PHA in comparison to control values at day seven.

Finally, we evaluated the cytokine concentration profile from the supernatant of PBMC cultures after seven days of exposure with the MT antigens and PHA (Fig. 3). Two

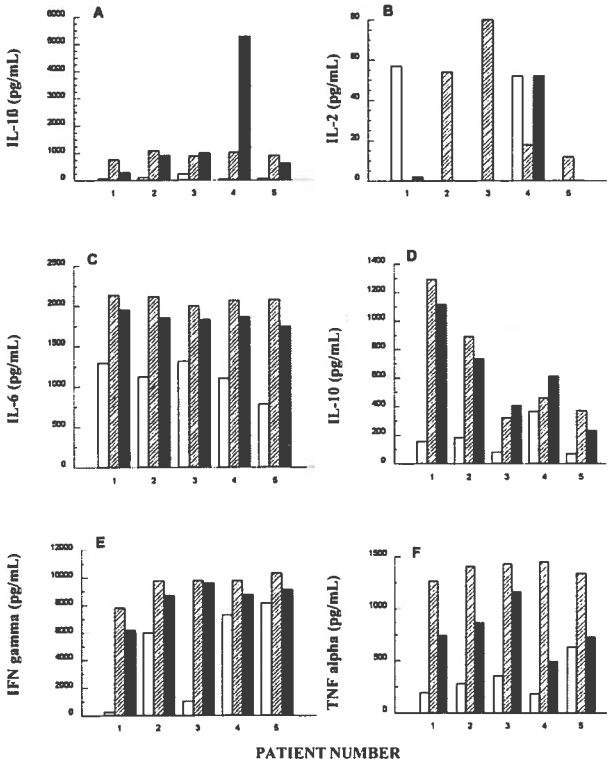

Figure 3. Cytokine profile in the supernatant of PMBC cultures from studied subjects after seven days exposure to MT antigens and PHA. The concentration of the following cytokines was determined: A) IL-1β, B) IL-2 , C) IL-6, D) IL-10, E) IFN-γ, and F) TNF- α. Patient no.1 had CML and active pulmonary tuberculosis; patients no. 2-5 had active pulmonary tuberculosis and no coexistent diseases. The results of unstimulated PBMC, (open bars), MT antigens-stimulated PBMC (hatched bars), and PHA-stimulated PBMC (solid bars) are shown.

important differences were noticed between the cell cultures from the CML patient and from the other tuberculosis patients. First, IL-2 was not detected when the PBMC from the CML patient were stimulated with MT antigens (Fig. 3B). This finding was in sharp contrast with the significant production of IL-2 observed for the control patients. Second, a high concentration of IL-10 (five to six-fold increase) was seen in response to MT antigens and PHA in the cell cultures from the CML patient (Fig. 3D). In addition, there was a mild reduction of IL-1β and INF-γ in response to MT antigens and PHA stimuli for the CML patient (Fig. 3A, 3E) The cytokine pattern for IL-6 and TNF-α was similar for all patients (Fig. 3C, 3F). IL-4 was undetected in all cell cultures (data not shown).

Discussion

This study was designed to gain a better understanding of the immunopathogenic mechanisms involved in CML that confer susceptibility to MT infection. Consistently throughout the cellular function studies, the PBMC from the CML and active tuberculosis patient exhibited, in the presence of MT antigens and PHA, a marked digressing pattern of immunological response from that observed in the group of patients with tuberculosis solely. The immunologic reactivity upon stimulation with MT antigens of the PBMC derived from the patients with pulmonary tuberculosis and no coexistent diseases was the expected response. In contrast, the PBMC from the CML patient did not proliferate in response to mycobacterial antigens. Specifically, the *Mycobacterium*-reative CD4+ T cells, Vß8 T cells and V-δ2 T failed to expand upon antigenic stimulation. Moreover, IL-10 was significantly produced by CML PBMC after mycobacterial stimulation. On the other hand, IL-2 was not produced and mild reduction of IL1-ß and INF-γ were observed. Similar immunologic abnormalities were observed with PHA stimulation for the CML patient.

The abnormalities of the cell-mediated immunity reported in this study are consistent with previous studies in CML. Gong et al. evaluated the antigen presenting capability of peripheral blood monocytes from 13 patients with CML by the isotope incorporation technique and found an impaired capability for all patients (11). In addition, they found a significant decrease in HLA-DQ+ monocytes. Bhatia et al studied the effect of T cells derived from bone marrow of CML patients on hematopoietic progenitors (12). Incubation of CML T cells with autologous bone marrow mononuclear cells resulted in suppression of hematopoietic progenitors. This suppressive effect was mediated by CD4+ T cells. Taken together, these studies demonstrate that CML patients have a significant immunological dysfunction that lowers their resistance to opportunistic infections in which a competent cellular immunity is required, such as mycobacterial, fungal and parasitic infections.

A plausible interpretation of the data presented in this work is that the decreased proliferative index shown by the CML patient is due to a reduced growth response of the Th1 cells in a cytokine environment that favors the predominance of Th2 cells (reduced production of IFNγ and IL-2, increased production of IL-10) and disease progression. Surprisingly, there was no distinctive production of IL-4 and IL-6, which would have better correlated with our proposed interpretation, since an abnormal pathological evolution, such as that presented by the patient with CML, has been associated with the production of endogenous products, most notable IL-1, IL-4 and IL-6, and TNF (19). However, there are conflicting reports indicating that IL-4 enhances and other that inhibits T cell proliferation (20-21), suggesting that the exact role of this and other cytokines as markers of disease pathogenesis needs further investigation.

The finding that IL-10 was significantly produced by the CML PBMC upon stimulation with MT antigen and PHA is very important. IL-10, an inhibitory interleukin produced by macrophages and lymphocytes, affects most proinflammatory cytokines (22). IL-10 suppresses cytokine synthesis by monocytes and inhibits antigen-mediated T cell proliferation (23-24). Likewise, IL-10 exerts a potent inhibitory effect on IL-12 synthesis (25). IL-12 enhances the proliferation of Th1 cells and stimulates the production of INF-γ (26), both important events in conferring immunity against *Mycobacterium tuberculosis*. Thus, it is possible that IL-10 had an important role in the lack of expansion of *Mycobacterium*-reactive T cells and decreased production of proinflammatory cytokines observed in this study by the CML PBMC.

It can be argued that the abnormal immune response observed in the CML patient is due to the immunosuppression caused by tuberculosis and not by leukemia. Several reports demonstrate that patients with active tuberculosis may have abnormal cell-mediated immunity such as anergy on MT skin tests and decreased proliferative response and cytokine production upon MT stimulation (27-30). In a population of active tuberculosis patients, Sánchez et al. reported similar findings to our patient with CML (31). They found decreased proliferative responses to purified protein derivative (PPD), no expansion of γδ T cells and decreased IL-2 and IFN-γ after stimulation with PPD. We do not think, however, that TB-mediated immunosupression was an important

factor in our CML patient. Most of these previous studies were performed in untreated patients or in the first few weeks of treatment. In this phase of infection the immune abnormalities are greater. In our study, all patients were on their second to third month of treatment. Also, at the time of experimental studies, the duration of tuberculosis infection, clinical manifestations, and modality of anti-tuberculosis treatment were similar for all patients. Furthermore, the pattern of the MT-induced proliferative response, T cell subpopulation expansion and cytokine production was almost identical for the four patients with tuberculosis and no CML. None of the immulogic disturbances reported by Sánchez et al. were seen in the control patients.

The immunosuppression observed in cancer patients could be further aggravated by the use of anti-tumor agents. In this study, the CML patient was receiving treatment with hydroxyurea which is an inhibitor of ribonucleotide reductase. Although this drug causes inhibition of DNA synthesis, it should not alter RNA or protein synthesis. Certainly, hydroxyurea could have an effect on the proliferative response in the CML patient, but is unclear whether it could have any significant effect in other cellular functions. Recent studies in HIV-infected patients suggest a possible immunomodulatory effect of hydroxyurea. HIV-infected patients treated with hydroxyurea and didanosine showed suppressed HIV-production in resting and activated PBMC, which suggests a possible role of hydroxyurea in cellular immunosuppression (32-34). Therefore, this drug could have a potential role in the cellular abnormalities observed in the CML patient.

Experimental studies, such as the present, addressing the specific immunologic abnormalities in immuno-compromised patients have important therapeutic implications. In the management of MT infection, pharmacologic treatment or chemotherapy is the most important. However, the utilization of immunomodulating agents, particularly in immunosuppressed patients might be very effective. For example, vaccine preparations that induce or expand Th1 cells or cytokines can further strengthen the immune response to mycobacteria. Similarly, administration of proinfammatory and tubericidal cytokines such as IL-2 and INF-γ could also be beneficial. Finally, the marked production of IL-10 observed in this study could be neutralized by antibodies or drugs that decrease the synthesis of this cytokine.

The present work indicates that PBMC from a patient having concomitant active pulmonary tuberculosis and CML have a reduced proliferative capacity, lack of expansion of *Mycobacterium*-reactive T cells and abnormal cytokine secretion in response to mycobacterial antigens. A definite alteration of the Th1/Th2 ratio, with a net balance favoring predominance of Th2 cells was observed. It also emphasizes the importance of developing further efforts to correct these immunological dysfunctions that could improve the general health of patients having CML and other immunosuppressive conditions.

Resumen

Los mecanismos patogénicos de inmunosupresión en leucemia crónica mielocítica (LCM) que conllevan a una susceptibilidad a infección con *Mycobacteriun tuberculosis* (MT) no están claros. Para evaluar este punto, medimos la respuesta proliferativa, variación de subpoblaciones de linfocitos T (CD4+, CD8+, TCR-Vδ2 y TCR-Vß8) y perfil de citocinas (IL-1β , IL-2, IL-4, IL-6, IL-10, TNF-α y IFN-γ) luego de estimular con MT células mononucleares de sangre periférica (CMSP) de un paciente con LCM y tuberculosis activa concomitante. Los resultados fueron comparados con cuatro pacientes con tuberculosis activa y sin otras enfermedades coexistentes. La respuesta inmunológica a fitohemaglutinina (FHA) también fue evaluada. En contraste a los controles, los CMSP de LCM fueron incapaces de proliferar a los antígenos de MT. Células T CD4+, Vδ 2 y Vß8 reactivas *a Mycobacterium* no se expandieron luego de estimulación con MT en CMSP de LCM. Cultivos estimulados con MT del paciente con LCM no produjeron IL-2 y se observó una reducción leve de IL-1ß y INF- γ. En contraste, IL-10 se elevó marcadamente en estos cultivos. De forma similar, CMSP del paciente con LCM estimulados con FHA no demostraron expansión de células T CD4+ y CD8+. En estos cultivos la concentración de INF-γ en los sobrenadantes estaba disminuida y la de IL-10 significativamente elevada. Este estudio sugiere que pacientes con LCM pueden presentar una inmuno-supresión profunda de efectores celulares y moleculares esenciales, creando un escenario que podría contribuir al desarrollo de tuberculosis activa. Estos hallazgos apoyan la nesecidad de establecer modalidades inmunotera-péuticas con valor potencial para los desórdenes mieloproliferativos.

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