Modulation of Immune Response by the Acute and Chronic Exposure to High Altitude

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ABSTRACT

FACCO, M., C. ZILLI, M. SIVIERO, A. ERMOLAO, G. TRAVAIN, I. BAESSO, S. BONAMICO, A. CABRELLE, M. ZACCARIA, and C. AGOSTINI. Modulation of Immune Response by the Acute and Chronic Exposure to High Altitude. *Med. Sci. Sports Exerc.*, Vol. 37, No. 5, pp. 768–774, 2005. **Purpose:** The chronic exposure at high altitude (HA) represents an ideal model for evaluating the *in vivo* effects of hypobaric hypoxia. Taking advantage of the EV-K2-CNR Pyramid, this study was designed to evaluate whether acute and chronic hypoxia differently modulates the *in vivo* immune responses. **Methods:** The study includes 13 healthy female moderately active volunteers participating to the Italian HA project EV-K2-CNR. Peripheral blood lymphocytes, collected at sea level and at HA in the Pyramid Laboratory of CNR, Nepal (5050 m), were immunologically characterized by flow cytometry and a series of molecular and functional analyses. **Results:** Flow cytometric analyses showed that: a) $CD3^+$ T lymphocytes significantly decreased during both acute and chronic exposure to HA, b) T-cell fall was totally due to $CD4^+$ T-cell reduction, c) B lymphocytes were not influenced by the exposure to HA, and d) natural killer (NK) cells significantly increased during acute and chronic exposure. The evaluation of the Th1/Th2 pattern demonstrated a significant decrease of the expression of the Th1 cytokine interferon-γ (IFN-γ) by circulating T cells during acute and chronic exposure to HA. The expression by T cells of CXCR3, a chemokine receptor typically expressed by Th1/Tc1 cells, paralleled the decrease of IFN-γ. On the contrary, the expression of IL-4 was not conditioned by the exposure to HA. Finally, functional studies showed a significant reduction of the proliferative activity in response to mitogen (PHA) both in acute and chronic HA exposure. Despite the increased number of NK cells, NK cytotoxic activity was not influenced by the HA exposure. **Conclusions:** Our results indicate that the *in vivo* exposure to HA leads to an impairment of the homeostatic regulation of Th1/Th2 immune balance that potentially could favor long-term immunological alterations and increase the risk of infections. **Key Words:** TH1 CELLS, TH2 CELLS, LYMPHOCYTES, CYTOKINES, CHEMOKINES, ENVIRONMENT

A number of data support the relationship between immunity and environment as a result of the interaction between immune and neuroendocrine systems. Environmental conditions (temperature, hypoxia, physical exercise) represent some of the stimuli that may influence the functional behavior of immunocompetent cells (3,8,15). Although several efforts have been devoted to understand the effects of the impact of extreme environmental conditions on respiratory, cardiovascular, and musculoskeletal systems, less known is the adaptive capability of the immune system to different adverse environmental conditions. Specific parameters, including evaluation of lymphocyte subsets and cytokine expression in relationship to hormone production and strenuous exertion have been considered (14,16). Other groups have investigated the effects of hypoxia on modulating different activities of immunocompetent cells as well as their capability to produce cytokines (5). As an example, it has been recently demonstrated that lymphocytes, and cytotoxic cells show a peculiar adaptation to hypoxic stimuli, leading to different patterns of lymphokine secretion and accumulation (4). However, there is general agreement that T cell-mediated immunity is impaired from hypoxia, whereas NK cells are likely to be responsive only with transitory changes (15).

Most of these above data have been obtained by *in vitro* studies, although the *in vivo* effects of hypobaric hypoxia on immune system need to be further elucidated. High altitude (HA) permanence (up to 5000 m) represents an ideal model to evaluate the *in vivo* effects of hypobaric hypoxia on the immune system. Taking advantage of the EV-K2-CNR Pyramid, this study was designed to evaluate whether acute and chronic hypoxia differently modulates the *in vivo* immune phenomena. Specifically we evaluated the capability of T cells to produce different patterns of cytokines and their functional activity in response to high altitude, evaluating the hypothesis that high altitude exposure, as acute and chronic stress condition, may *in vivo* affect specific functions of cellular immune response, favoring an immunodeficiency condition that increases the risk of infections.

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TABLE 1. Values of the peripheral white blood cells in 13 normal female subjects.

	White Blood Cells				Lymphocytes							
					SL_1		P_1		P_2		SL_2	
Subject No.	SL_1 (10^3 mL)	P_1 (10^3 mL)	P_2 (10^3 mL)	SL_2 (10^3 mL)	10^3 mL	%	10^3 mL	%	10^3 mL	%	10^3 mL	%
1	6.3	8.0	5.4	10.0	2.2	34.9	1.7	21.6	2.1	38.0	3.0	30.0
2	8.3	8.6	8.6	6.4	2.2	26.5	1.7	20.0	3.9	45.5	1.6	25.6
3	7.2	4.0	10.8	3.5	1.4	19.3	0.6	15.0	2.2	20.4	0.8	22.3
4	6.1	5.8	7.2	3.2	2.4	39.3	1.3	22.0	3.3	46.4	0.7	21.5
5	5.0	4.2	5.8	6.0	2.3	46.0	1.3	31.3	2.2	38.8	1.5	24.8
6	5.0	8.4	7.2	5.0	1.4	28.0	1.7	20.1	2.6	36.6	1.0	20.2
7	4.2	4.8	9.2	10.4	1.7	40.5	0.7	16.5	2.3	25.2	1.9	18.6
8	5.6	9.2	10.1	6.5	2.5	43.5	2.7	29.8	2.4	24.3	2.4	36.2
9	5.7	7.3	6.3	6.4	1.3	22.5	1.0	13.5	2.0	32.3	1.7	27.2
10	6.9	6.7	7.0	7.0	2.7	39.1	0.6	9.4	2.7	38.0	2.1	29.9
11	6.8	6.5	6.7	4.5	2.7	39.7	1.9	29.4	2.1	31.1	1.8	38.8
12	5.4	4.2	7.6	2.8	1.8	33.3	0.7	16.8	1.6	20.9	0.9	30.7
13	5.5	6.5	7.1	6.9	1.5	27.3	1.5	23.4	2.2	30.4	2.0	28.7
Mean	6.0	6.5	7.6*	6.0	2.0	33.8	1.3*	20.7*	2.4	32.9	1.6	27.3
SD	1.1	1.8	1.6	2.3	0.5	8.4	0.6	6.6	0.6	8.6	0.6	6.0

Comparison among SL_1, P_1, P_2, and SL_2 detections as follows: * $P < 0.05$.

MATERIALS AND METHODS

Study population. Thirteen healthy moderately active young women (aged 21.3 ± 3.1 yr) participated in the Italian high altitude project EV-K2-CNR. All subjects flew from Kathmandu (1300 m, Nepal) to Lukla (2866 m) and then trekked for 5 d until reaching the altitude of 5050 m (Pyramid Laboratory of Italian Research Council, CNR). The volunteers remained in the laboratory the following 21 d, carrying out their standard degrees of sea-level physical activity. In fact, all the subjects were exercise science students, involved in regular, noncompetitive, moderate-intensity exercises, performed for 90 min, 3–5 $d \cdot wk^{-1}$. During the permanence at high altitude, they maintained the standard level of physical activity, by short treks, indoor aerobic and strength exercises, performed for 60–90 min, 3–5× wk^{-1}, always maintaining a moderate intensity. Sixty milliliters of peripheral blood samples were collected at the same time in the morning (8:00 a.m.), at sea level (SL_1, the day before departure; SL_2, the day after returning) in the laboratory of the Sport Medicine Unit of Padua University (12 m), and at HA in the Pyramid Laboratory of CNR, on Lobuche, Nepal (5050 m) (P_1, the first day at 5050 m; P_2, the 21st and last day at 5050 m). Blood was drawn from the antecubital vein of the fasted and supine volunteers. The experiments comply with the current laws of Italy and Nepal. Written informed consent was obtained from each participant.

Recovery and handling of peripheral blood cells. Peripheral blood mononuclear cells (PBMC) from subjects under study were obtained from freshly heparinized blood after centrifugation on a Ficoll–Hypaque gradient, washed with phosphate-buffered saline (PBS), resuspended in medium RPMI-1640 (Gibco Laboratories, Grand Island, NY), and counted in a hemocytometer. A differential count of macrophages, lymphocytes, neutrophils, and eosinophils (made from total counts of 300 cells) was carried out on cytocentrifuged smears stained with Wright–Giemsa. The cells were then stored frozen at −70°C until the return to sea level.

Phenotypic evaluation. PBMC from each subject were characterized using different mAb, including those belonging to CD3, CD4, CD8, CD16, CD19, and CD56

TABLE 2. Flow cytometry analyses of peripheral T cell subsets in 13 normal female subjects. The frequency of lymphocytes positive for mAb was determined on 10,000 cells bearing typical lymphocytes scatter; CD3, CD4, and CD8 expression of normal subjects, in normoxia condition, ranges, respectively, from 70 to 80%, from 40 to 54%, and from 22 to 34%.

Subject No.	CD3 T Lymphocytes (%)				CD4 T Lymphocytes (%)				CD8 T Lymphocytes (%)			
	SL_1	P_1	P_2	SL_2	SL_1	P_1	P_2	SL_2	SL_1	P_1	P_2	SL_2
1	73.0	60.0	67.0	90.0	49.0	36.0	47.0	73.0	21.0	26.0	15.0	15.0
2	77.0	61.0	57.0	74.0	54.0	32.0	32.0	57.0	18.0	23.0	27.0	19.0
3	69.0	58.0	53.0	77.0	48.0	40.0	38.0	59.0	20.0	19.0	18.0	20.0
4	65.0	31.0	32.0	67.0	47.0	8.0	23.0	47.0	17.0	20.0	15.0	21.0
5	69.0	50.0	53.0	66.0	47.0	33.0	37.0	50.0	22.0	21.0	18.0	18.0
6	84.0	65.0	65.0	85.0	56.0	36.0	35.0	58.0	25.0	30.0	31.0	24.0
7	74.0	60.0	29.0	78.0	55.0	37.0	11.0	51.0	22.0	26.0	20.0	27.0
8	70.0	56.0	54.0	77.0	49.0	39.0	34.0	56.0	20.0	19.0	21.0	19.0
9	69.0	52.0	49.0	71.0	45.0	31.0	27.0	49.0	22.0	21.0	23.0	21.0
10	73.0	55.0	57.0	80.0	49.0	30.0	32.0	55.0	23.0	26.0	24.0	27.0
11	79.0	58.0	44.0	74.0	49.0	34.0	27.0	48.0	27.0	26.0	25.0	25.0
12	65.0	44.0	49.0	71.0	48.0	26.0	33.0	50.0	18.0	21.0	19.0	22.0
13	86.0	59.0	62.0	85.0	59.0	35.0	40.0	59.0	24.0	26.0	23.0	25.0
Mean	73.3	54.5*	51.6*	76.5	50.4	32.1*	32.0*	54.8	21.5	23.4	21.5	21.8
SD	6.6	8.9	11.4	7.1	4.2	8.2	8.8	7.0	2.9	3.5	4.7	3.6

Comparison among SL_1, P_1, P_2, and SL_2 detections as follows: * $P < 0.05$.

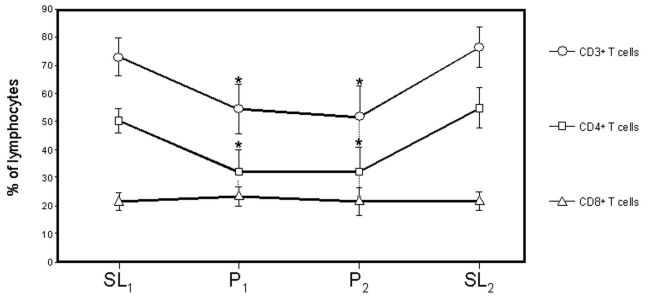

FIGURE 1—Changes in T lymphocytes related to the exposure to high altitude. Total $CD3^+$ T cells showed a significant decrease during both acute (P_1) and chronic (P_2) exposure to high altitude. The reduction of T population was totally ascribed to $CD4^+$ T-cell fall. SL_1: the day before departure, at sea level; P_1: the first day at 5050 m; P_2: the 21st and last day at 5050 m; SL_2: the day after returning, at sea level; * $P < 0.05$.

(Becton Dickinson, Sunnyvale, CA). Cells were further characterized with mAb anti-IL-4, anti-IFN-γ (PharMingen, San Diego, CA), and antihuman CXCR3 (R&D Systems Inc., Minneapolis, MN). Cells were studied for the expression of cell surface antigens with direct two-color analysis using FITC-conjugated and phycoerythrin-conjugated mAb using flow cytometry analysis (FACScan, Becton Dickinson) as previously described (6). Expression of the cytoplasmic cytokines was evaluated after permeabilization of the cell membranes with 1:2 diluted PermeaFix (Ortho, Raritan, NJ) for 40 min. After permeabilization procedures, anti-IL-4 and anti-IFN-γ mAb were added. Cells were analyzed using a FACScan analyzer (Becton Dickinson), and data were processed by using the CellQuest program. Ten thousand cells bearing the typical lymphocyte scatter were scored.

RNA extraction, cDNA synthesis, and PCR amplification of IFN-γ, IL-4, and CXCR3. Total cellular RNA was extracted using the Ultraspec I RNA isolation system (Biotecx Lab., Houston, TX) from PBMC. cDNA were prepared from 2 μg of total cellular RNA by reverse transcription (RT) using a kit from Invitrogen Corp. (San Diego, CA). For the amplification of IFN-γ, IL-4, and β-actin, PCR was conducted in 50-μL reaction using 2 μL of cDNA as a template. The PCR mixture consisted of 1.5 $mmol \cdot L^{-1}$ $MgCl_2$, 50 $mmol \cdot L^{-1}$ KCl, 10 $mmol \cdot L^{-1}$ Tris-HCl, 0.2 $mM \cdot L^{-1}$ concentrations of each deoxynucleotide triphosphate, 2.5 U of Taq polymerase (Perkin Elmer, Norwalk, CT), and 25 pM of each specific primer. The following sense and antisense oligonucleotide primer sequences were used: for IFN-γ: 5'-AgT TAT ATC TTg gCT TTT CA, 3'-ACC gAA TAA TTA gTC AgC TT (expected size of 355 bp); for IL-4:5'-CTT CCC CCT CTg TTC TTC CT, 3'-TTC CTg TCg AgC CgT TTC Ag (expected size of 317 bp); for CXCR3: 5'-CAA CgC CAC CCA CTg CCA ATA CAA, 3'-CAg gCg CAA gAg CAg CAT CCA CAT (expected size of 415 bp); for β-actin: 5'-gTg ggg CgC CCC Agg CAC CA, 3'-CTC CTT AAT gTC ACg CAC gAT TTC (expected size of 540 bp). IFN-γ, IL-4, and CXCR3 were amplified at the following conditions: 60-s melting at 94°C, 45-s annealing at 53°C for IFN-γ, 58°C for IL-4, and 56°C for CXCR3, and 120-s extension at 72°C for 30 cycles followed by a final extension for 7 min at 72°C in a Cetus/Perkin Elmer thermal cycler. For β-actin amplification, conditions were 60-s melting at 94°C, 60-s annealing at 55°C, and 120-s extension at 72°C for 30 cycles followed by a final extension for 7 min at 72°C; 10 μL of each PCR product was electrophoresed in a 2% agarose gel in Tris borate/EDTA buffer. Gels were stained with ethidium bromide and photographed.

Proliferative activity. PBMC from four subjects at the concentration of 1×10^6 $cells \cdot mL^{-1}$ were cultured in 96-well round bottom plates (Corning, New York, NY) for 72 h at 37°C in 5% CO_2 modified atmosphere with phytohemagglutinin (PHA) (0.6, 2.5, and 5.0 $\mu g \cdot mL^{-1}$). Each experiment was performed in triplicate. For the last 18 h of culture, plates were pulsed with 0.037 Mbq (1 μC) per well of (H^3)thymidine (6.7 $Ci \cdot mmol^{-1}$, Du Pont, NEN, Boston, MA); cells were then harvested and the radioactivity measured with a beta counter. Results are expressed as counts per minute ± SD.

Cytotoxic activity. Cytotoxic activity was assessed by the lysis of ^{51}Cr-labeled NK-sensitive K-562 cells in a 4-h

TABLE 3. Flow cytometry analyses of peripheral B and NK lymphocytes in 13 normal female subjects. The frequency of lymphocytes positive for mAb was determined on 10,000 cells bearing typical lymphocytes scatter; the percentage of B and NK cells in normal subjects, in normoxia condition, ranges, respectively, from 7.0 to 13.0%, and from 8.0 to 18.0%.

Subject No.	B Lymphocytes (%)				NK Lymphocytes (CD16%)				NK Lymphocytes (CD56%)			
	SL_1	P_1	P_2	SL_2	SL_1	P_1	P_2	SL_2	SL_1	P_1	P_2	SL_2
1	9.0	11.0	11.0	7.0	13.0	21.0	17.0	7.0	7.0	18.0	17.0	9.0
2	14.0	14.0	16.0	13.0	11.0	25.0	20.0	14.0	8.0	22.0	22.0	10.0
3	10.0	9.0	11.0	9.0	21.0	27.0	28.0	16.0	19.0	25.0	23.0	17.0
4	9.0	11.0	13.0	8.0	18.0	25.0	23.0	18.0	7.0	38.0	23.0	6.0
5	9.0	13.0	13.0	12.0	19.0	25.0	24.0	18.0	19.0	27.0	21.0	17.0
6	6.0	5.0	5.0	4.0	11.0	28.0	28.0	15.0	18.0	28.0	25.0	13.0
7	4.0	10.0	8.0	8.0	15.0	23.0	20.0	17.0	15.0	35.0	27.0	14.0
8	11.0	14.0	11.0	10.0	10.0	29.0	19.0	14.0	10.0	37.0	28.0	12.0
9	8.0	9.0	9.0	9.0	19.0	27.0	24.0	18.0	6.0	29.0	29.0	7.0
10	10.0	11.0	9.0	8.0	17.0	24.0	20.0	16.0	13.0	28.0	28.0	18.0
11	13.0	14.0	11.0	13.0	8.0	20.0	20.0	11.0	20.0	28.0	26.0	12.0
12	8.0	7.0	7.0	9.0	7.0	26.0	11.0	10.0	12.0	25.0	25.0	21.0
13	4.0	10.0	8.0	6.0	15.0	25.0	24.0	12.0	9.0	26.0	26.0	9.0
Mean	8.8	10.6	10.1	8.9	14.1	25.0*	21.4*	14.3	12.5	28.1*	24.6*	12.7
SD	3.0	2.7	2.9	2.6	4.2	2.6	4.6	3.4	5.1	5.7	3.3	4.5

Comparison among SL_1, P_1, P_2, and SL_2 detections as follows: * $P < 0.05$.

assay, as previously reported (19). In all instances, target cells were used at a concentration of 10×10^4 mL^{-1} and the results referred to the 5:1, 10:1, 20:1, and 40:1 effector (E)/target (T) ratio. Each experiment was performed in triplicate.

Statistical analysis. Data were analyzed with the assistance of the Statistical Analysis System. Data are expressed as mean ± SD. Mean values were compared using the ANOVA test. A P value less than 0.05 was considered as significant.

RESULTS

Morphological analyses. Morphological features of white blood cells (WBC) obtained from the 13 subjects are reported in Table 1. The analyses performed on the blood samples collected during the four main periods (SL_1, P1, P_2, and SL_2) showed a significant increase of WBC during the chronic exposure (P_2, 3 wk at high altitude). On the contrary, the lymphocytes were characterized by a sensible decrease during the acute exposure (P_1, 5 d of climbing from 2866 to 5050 m). WBC and lymphocytes recuperated the original values when the subjects returned to sea level (SL_2) (Table 1).

Phenotypical analyses of lymphocyte cells. To define the variations on lymphocytes related to the exposure to high altitude, flow cytometry analyses were carried out on T, B, and NK lymphocyte populations (Table 2). The studies performed on $CD3^+$ T cells showed a significant decrease of T lymphocytes during both acute and chronic exposure to high altitude. In particular, the reduction of T population was totally caused by $CD4^+$ T-cell fall, without any significant change in $CD8^+$ T cell subset values (Fig. 1).

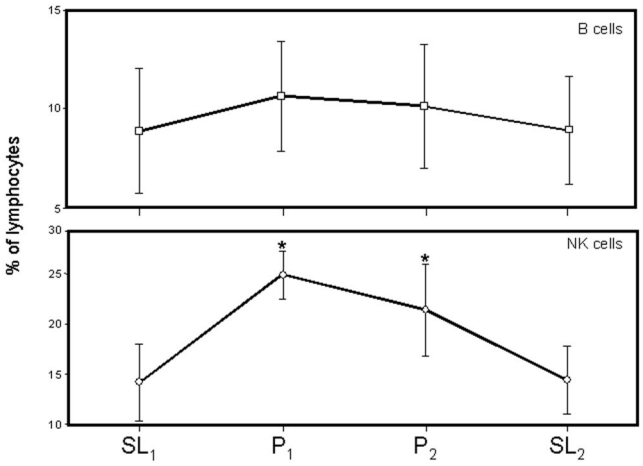

FIGURE 2—Data obtained from flow cytometry analyses on B and NK cells: whereas B lymphocytes were not influenced by the exposure to high altitude, NK cells were significantly increased during acute (P_1) and chronic (P_2) exposure. SL_1: the day before departure, at sea level; P_1: the first day at 5050 m; P_2: the 21st and last day at 5050 m; SL_2: the day after returning, at sea level; * $P < 0.05$.

Table 3 showed the data obtained from the analyses on B and NK cells: whereas B lymphocytes were not influenced by the exposure to high altitude, NK cells were significantly increased during acute and chronic exposure (Fig. 2).

Each lymphocyte population had recovered normal values when the subjects returned to sea level.

IFN-γ, IL-4, and CXCR3 expression on T lymphocytes. Using phenotypical and molecular approaches, we evaluated the cytokine pattern of T cells and the chemokine receptor CXCR3. CXCR3 binds the molecules CXCL9/Mig, CXCL10/IP-10, and CXCL11/Itac:

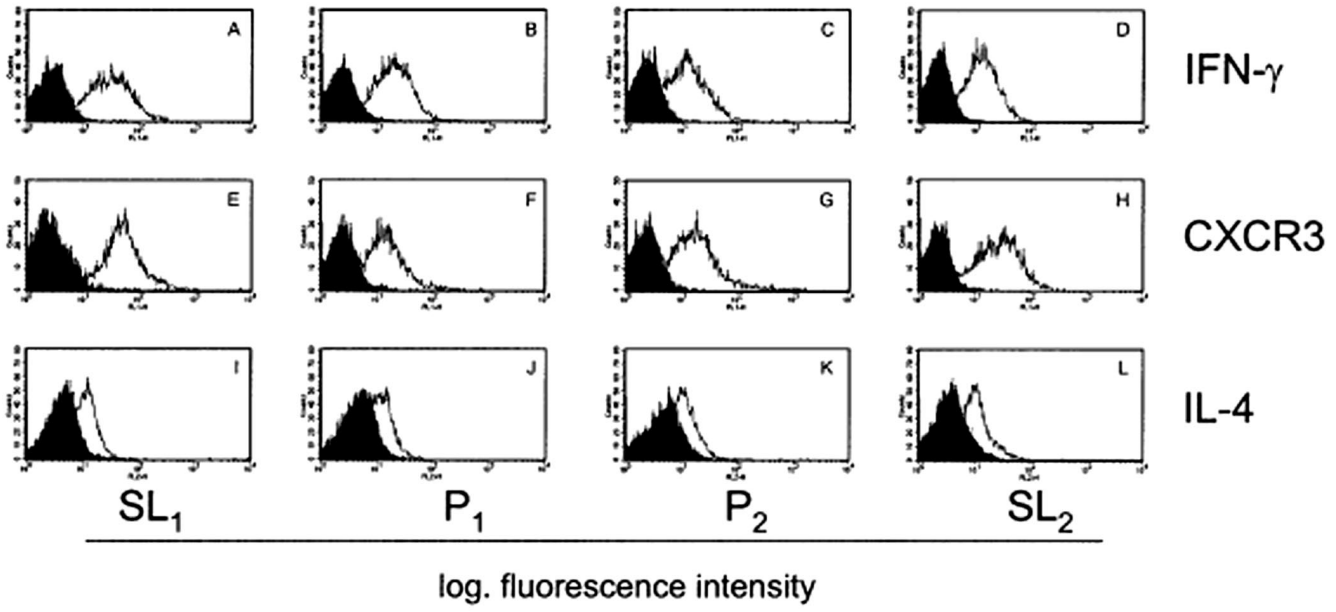

FIGURE 3—Data obtained from flow cytometry analyses on T cells. T lymphocytes expressed IFN-γ (Fig. 3, A–D). The expression of this cytokine significantly decreased during acute (P_1) and chronic (P_2) exposure to high altitude, and it was not recovered even when the subjects returned to sea level (SL_2) Interestingly, CXCR3 (Fig. 3, E–H) displayed the same decreasing rate. The expression of IL-4 (Fig. 3, I–L) was not conditioned to both the acute (P_1) and the chronic (P_2) exposure to high altitude, and it did not show any significant change until the return to sea level. SL_1: the day before departure, at sea level; P_1: the first day at 5050 m; P_2: the 21st and last day at 5050 m; SL_2: the day after returning, at sea level.

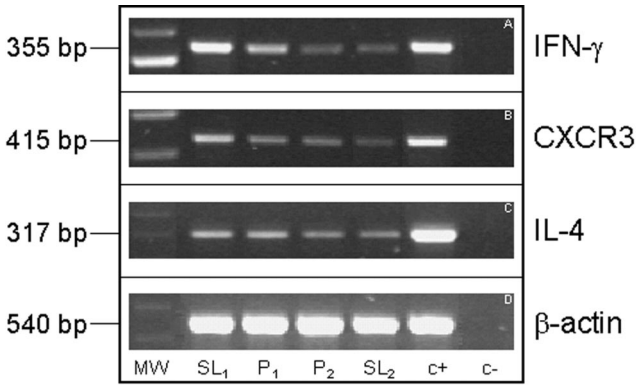

FIGURE 4—RT-PCR analyses on T cells. A) IFN-γ mRNA expression. B) CXCR3 mRNA expression. C) IL-4 mRNA expression. D) β-actin mRNA expression. SL_1: the day before departure, at sea level; P_1: the first day at 5050 m; P_2: the 21st and last day at 5050 m; SL_2: the day after returning, at sea level.

the production and the release of these chemokines are induced by IFN-γ (7,11).

T lymphocytes expressed IFN-γ, a typical Th1/Tc1 cytokine (Fig. 3, A–D). The expression of this cytokine significantly decreased during acute and chronic exposure to high altitude, and it was not recovered even when the subjects returned to sea level (MFI average from 13 subjects: 32.0 ± 8.5 at SL_1, 19.2* ± 5.5 at P_1, 7.0* ± 3.7 at P_2, and 3.0* ± 2.1 at SL_2; $*P < 0.05$). Interestingly, CXCR3 displayed the same decreasing rate (MFI average from 13 subjects: 69.0 ± 35.3 at SL_1, 35.7* ± 17.6 at P_1, 29.6* ± 16.5 at P_2, and 21.4* ± 16.0 at SL_2; $*P < 0.05$) (Fig. 3, E-H). On the contrary, the expression of IL-4 was not conditioned to both the acute and the chronic exposure to high altitude, and it did not show any significant change until the return to sea level (MFI average from 13 subjects: 7.0 ± 4.2 at SL_1, 6.0 ± 6.4 at P_1, 4.3 ± 3.1 at P_2, and 5.1 ± 3.3 at SL_2) (Fig. 3, I-L). mRNA analyses confirmed the different changes of IFN-γ (Fig. 4A), CXCR3 (Fig. 4B), and IL-4 (Fig. 4C), related to mRNA of β-actin (Fig. 4D).

Proliferative activity. To evaluate whether the proliferative activity of PBMC could be affected by the exposure to high altitude, the cells were cultured in the presence of PHA, an aspecific mitogen for T lymphocytes. The tests performed on the blood samples collected during the four main periods showed a relevant and significant reduction of the proliferative activity of the cells starting at acute (P_1) and enduring through chronic exposure (P_2) until the return to sea level (SL_2). Figure 5 shows a representative case (no. 8) of the four cases tested.

Cytotoxic activity. Although the proliferative activity of PBMC clearly suffered the exposure to high altitude, cell cytotoxicity was not influenced by this stressing condition. In fact, the cytotoxic activity did not show any significant increase or decrease during the different phases of the experiment. Figure 6 reported the cytotoxic activity of a representative case (no. 10) of the three cases tested.

DISCUSSION

In this article, we analyzed the effects of high altitude on white blood cells, in particular on the lymphocyte populations of 13 healthy young women. The analyses performed on samples collected at sea level (SL_1, the day before departure; SL_2, the day after returning) and at high altitude (P_1, the first day at 5050 m; P_2, the 21st and last day at 5050 m) showed a significant increase of WBC during the chronic exposure at high altitude (P_2). On the contrary, the lymphocytes were characterized by a significant decrease during the acute exposure (P_1): particularly, the phenotypical characterization of lymphocyte subsets demonstrated a significant decrease of T cells during both the acute and the chronic exposure (P_1 and P_2). Interestingly, the fall of T lymphocytes was completely ascribed to $CD4^+$ T-cell subset, whereas $CD8^+$ T lymphocytes did not show any significant variations. $CD4^+$ T cells recovered values in the normal range when the subjects returned to sea level.

It is known that exposure to high altitude and the consequent hypoxia as well as exposure to UVA may influence T cell immunity (10,15). Specifically, recent observations indicate that CD4+ skin T lymphocytes significantly diminish when exposed to UVA (12,18).

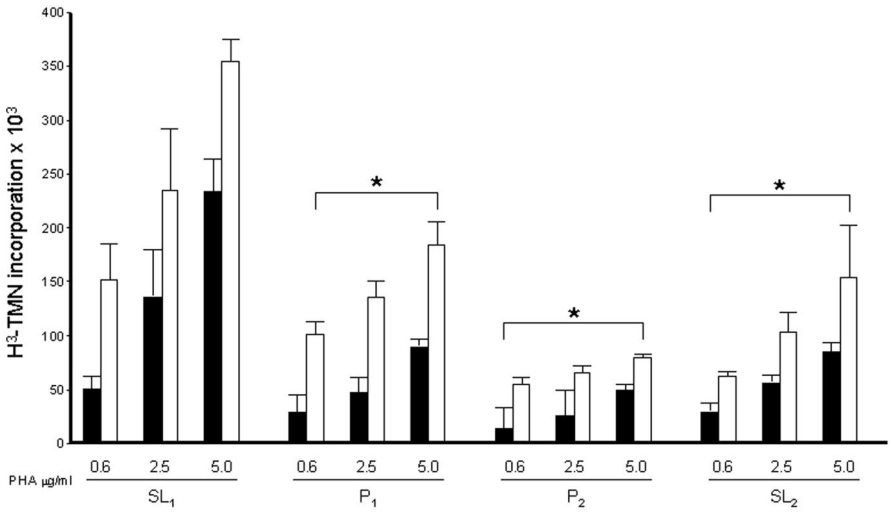

FIGURE 5—Proliferative activity of PBMC of a representative case (no. 8). The cells were cultured in the presence of PHA. The tests showed a significant reduction of the proliferative activity of cells starting at acute (P_1) and enduring through chronic exposure (P_2) until the return to sea level (SL_2). Figure 5 shows a representative case (no. 8) of the four cases tested. SL_1: the day before departure, at sea level; P_1: the first day at 5050 m; P_2: the 21st and last day at 5050 m; SL_2: the day after returning, at sea level; $* P < 0.05$.

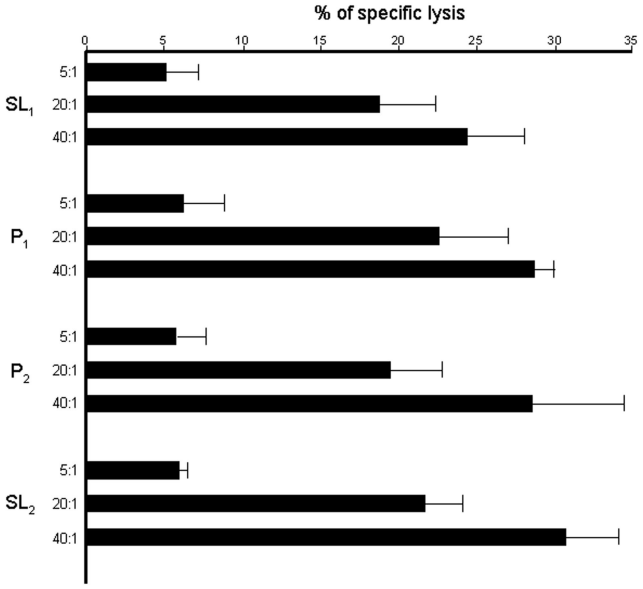

FIGURE 6—Cytotoxic activity of a representative case (no. 10). The activity did not show any significant change during the different phases of the experiment. SL_1: the day before departure, at sea level; P_1: the first day at 5050 m; P_2: the 21st and last day at 5050 m; SL_2: the day after returning, at sea level.

However, experiments performed in decompression chambers that simulate hypoxic conditions have clearly shown a decrease of circulating $CD4^+$ T cells (10). Taken together, these data suggest that both phenomena are likely to be involved in the rapid and persistent decrease of $CD4^+$ T cells in our subjects.

The exposure to high altitude did not affect B lymphocyte counts during the whole permanence period in the Pyramid Laboratory, whereas NK cells seemed to be particularly sensitive to the altitude and the hypoxia. In fact, cells bearing NK cell markers CD16 and CD56 showed a significant increase during permanence at high altitude, recovering to normal values only when volunteers returned to sea level. The evidence of the transient but sensible effect of high altitude and hypoxia on NK lymphocytes are in agreement with previous data obtained in subjects maintained in a decompression chamber, an experimental condition that mimics our *in vivo* conditions (10).

In another set of experiments, we evaluated whether numerical variations of lymphocyte subsets, principally T and NK cells, were associated with functional cell abnormalities. Our data demonstrate a differential pattern of cytokine production by T cells exposed to high altitude. In fact, during acute (P_1) and chronic (P_2) exposure to high altitude, T cells were characterized by a significant decrease of the expression of IFN-γ, that is, the paradigmatic Th1/Tc1 cytokine. The alteration of Th1 expression was maintained when subjects returned to sea levels (SL_2). On the contrary, the expression of the typical Th2/Tc2 cytokine IL-4 was not conditioned by high altitude and hypoxia exposure. Changes in Th1/Th2 or Tc1/Tc2 cytokine pattern have been described in mice subjected to different tensions of oxygen (4) and in athletes after exercise (17), in whom circulating Th1 cytokine-producing cells decrease more than Th2 cytokine-producing cells.

The alterations in the cytokine pattern of T cells in our volunteers were accompanied by significant changes in the expression of molecules Th1/Tc1. In fact, CXCR3, expressed by T cells, displayed a decreased rate similar to IFN-γ. CXCR3 is a receptor that belongs to the CXC superfamily of chemokines (2,9). It is selectively involved in the organ specific traffic of T lymphocytes (7,11), binding chemokines induced by IFN-γ (CXCL9/Mig, CXCL10/IP-10, and CXCL11/Mig) that are specifically chemotactic for activated T cells (1,13). Our data on CXCR3 expression are further confirmatory of a disruption in the homeostatic regulation of Th1/Th2 immune responses that could lead to potential long-term immunological alterations. However, the biological significance of the Th1/CXCR3 alteration induced by high altitude exposure remains unclear.

We also observed that proliferative activity of peripheral blood mononuclear cells was affected in the 13 volunteers: in fact, PBMC showed a significant reduction of cell proliferation, present in acute exposure (P_1) and maintained in phases of chronic exposure (P_2) until subjects returned to sea level (SL_2). The fall in T cell responsiveness to mitogenic stimulation (PHA) could be related to the decrease in Th1 population and, specifically, in IFN-γ and IL-2 producing cells. As a matter of the fact, recent data obtained in mice (4) have demonstrated that hypoxia inhibits not only IFN-γ but also IL-2 expression, because this Th1 cytokine is directly involved in T cell stimulation and proliferation. Although the proliferative activity was affected in our subjects, cell cytotoxicity appeared to be not influenced by the exposure to high altitude. There were no differences in this functional activity during the study period. Thus, NK cells that were significantly increased during both acute and chronic exposure are functionally working.

In summary, our results indicate that the exposure to high altitude and the consequent hypoxia are able to alter a number of cellular and functional immunologic parameters. Further studies in other subjects are needed to evaluate the physiologic significance of these changes, particularly in terms of chemokine and cytokine expression by T and NK cells. Furthermore, because our study population included only females, we are planning a new project aimed at evaluating whether there are gender differences in the homeostatic regulation of immune response in individuals exposed to high altitude.

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