Undersea & Hyperbaric Medicine, Vol. 20, No. 3, 1993

Effect of a single exposure to hyperbaric oxygen on blood mononuclear cells in human subjects

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Bitterman N, Bitterman H, Kinarty A, Melamed Y, Lahat N. Effect of a single exposure to hyperbaric oxygen on blood mononuclear cells in human subjects. Undersea & Hyperbaric Med 1993; 20(3):197–204.—We studied the effect of a single exposure to a therapeutic profile of hyperbaric oxygen on blood mononuclear cell subset. Twenty healthy volunteers were exposed to 0.28 MPa for 90 min. Thirteen breathed pure oxygen and seven were control subjects exposed to compressed air at the same pressure. Venous blood samples were drawn before HBO exposure, immediately on exit from the chamber, and 24 h later. Immediately after the exposure, a significant increase was observed in the percentage and absolute number of CD8 (suppressor/cytotoxic) T cells, with a concomitant decrease in the CD4 (helper/inducer) T cells. These changes resulted in a decreased CD4:CD8 ratio. A rise was also observed in the number of HLA–DR antigen-bearing cells, with a transient increase in monocytes. There was no change in the total count and percentage of T cells (CD3), B cells, and NK cells. Twenty-four hours after HBO exposure there was a partial reversal of the decrease in the mean CD4:CD8 ratio, but it was still significantly lower than preexposure values. The fast reversibility of the change in the CD4:CD8 ratio suggests specific HBO-induced shifts and sequestration of T-cell subpopulations.

hyperoxia, lymphocytes, immunosuppression, immune system

Hyperbaric oxygen is used for the treatment of decompression sickness (1), air embolism, carbon monoxide intoxication, gas gangrene, soft tissue infections, traumatic peripheral ischemia, skin grafts, etc. (2, 3). Immune competence is of major importance in these postsurgical, traumatic, and infectious conditions. HBO has also been suggested for the treatment of multiple sclerosis and other diseases related to autoimmune phenomena (4–6). Unfortunately, only limited information is currently available regarding the effect of a therapeutic HBO profile on the immune system in normal and pathologic conditions.

Several experimental studies have demonstrated immunosuppressive effects of repeated daily exposure of animals to HBO (7–11). Hansbrough et al. (7) reported that prolonged HBO exposures over a period of several days decreased circulating

leukocytes, spleen weight, and DNA synthesis in draining lymph nodes in mice. Other studies reported that cell-mediated reactions were suppressed by HBO, including reaction to tuberculin, adjuvant disease, allograft rejection, the onset of experimental allergic encephalomyelitis (8, 9), and the appearance of autoimmune symptoms (10). A decrease in interleukin-2 (IL-2) production (6), and in lymphocyte proliferation to mitogen stimulation (11) after repeated exposures to HBO, is further evidence of immunosuppressive effects in animal models.

Human studies seem contradictory. Feldmeier et al. (12) failed to demonstrate any effect of HBO on the immune response of healthy human volunteers treated over an extended period of time by daily HBO exposures to 0.24 MPa for 90 min. In contrast, Ginaldi et al. (13) reported reduced serum levels of a soluble IL-2 receptor and IL-2 after HBO treatment as well as elevated serum CD8 level, which generally point to suppression of immune responses. The immunosuppressive effect demonstrated in animals was not found in multiple sclerosis patients treated by a series of HBO sessions. In contrast, Nyland et al. (14) found that HBO had a stimulating effect on the immune response of patients with multiple sclerosis.

In addition to a possible dissimilarity between animal and human responses to HBO, different effects of HBO on the immune system might stem from the selection of various oxygen profiles and different durations of therapy. The purpose of the present study was to evaluate the effect of a single commonly used exposure profile to HBO (oxygen at 0.28 MPa for 90 min) on peripheral blood mononuclear cells in healthy human subjects.

MATERIALS AND METHODS

Twenty healthy volunteers participated in the study, 7 females and 13 males; their ages ranged from 19 to 47 yr. All were in good health and were not taking medications of any kind.

Hyperbaric oxygen protocol

Experiments were performed once a week, on the same day of the week and at the same time in the morning to avoid any circadian variation in the subpopulations of circulating lymphocytes (15). Two subjects were studied on each occasion. Peripheral venous blood was drawn at 0800 h for base-line measurements. The subjects then entered the multicompartment hyperbaric chamber. The experimental profile consisted of exposure to oxygen at 0.28 MPa for 90 min (with a 5-min air break between two 45-min oxygen sessions), while control subjects were breathing compressed air at the same pressure of 0.28 MPa for the same period of time. Subjects were randomly assigned to either an HBO or hyperbaric air group. Both experimental groups had the same age distribution. A second blood sample was drawn immediately on leaving the hyperbaric chamber and a third, 24 h later. Blood samples were also taken from a number of subjects 48 and 72 h after the HBO exposure.

Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells were isolated from heparinized venous blood by Ficoll–Hypaque (Pharmacia) density gradient centrifugation for 30 min at 1,600

rpm. Mononuclear cells were washed 3 times with phosphate buffered saline (PBS) containing 1% fetal bovine serum (FBS). Viability was assessed using trypan blue dye exclusion, and the cells were counted and brought to $10^7 \cdot ml^{-1}$ in PBS + 1% FBS.

Lymphocyte subpopulations

Cells ($10^6 \cdot 0.1\ ml^{-1} \cdot tube^{-1}$) were incubated with monoclonal antibodies (Becton Dickinson), diluted 1:20 at 4°C for 45 min, washed in the cold PBS, and then further incubated with fluorescein $F(ab')_2$ coupled goat anti-mouse IgG (Kallestad) for 45 min at 4°C in the dark. The cells were again washed twice and suspended in 100 µl PBS + 1% FBS. The percentage of fluorescent cells was established by reading at least 200 cells for each staining in a fluorescent microscope.

Statistical analysis

Repeated measures analysis of variance (ANOVA) was used to examine the differences between lymphocyte subpopulations before treatment, upon leaving the hyperbaric chamber, and 24 h later. The level of significance was determined at $P < 0.05$. If analysis showed a significant difference, specific comparisons were carried out by Wilcoxon's signed rank test.

RESULTS

Breathing compressed air at a pressure of 0.28 MPa for 90 min did not result in any significant variations in mononuclear cell populations measured in the peripheral blood of healthy volunteers immediately upon leaving the hyperbaric chamber and 24 h later ($P > 0.05$ in all groups in repeated measures ANOVA) (Table 1).

Exposure to a commonly used therapeutic profile of HBO (100% O_2 at a pressure of 0.28 MPa for 90 min) resulted in acute variations in the level of cell-mediated

Table 1: Blood Mononuclear Cell Populations in the Peripheral Blood of Control Subjects (n = 7) Exposed to 0.28 MPa Air for 90 min (mean ± SE)

	Base-line Control	On Exit From Hyperbaric Chamber	24 h Later
CD3, %	56.8 ± 4.1	62.2 ± 4.4	53.4 ± 3.1
CD4, %	51.4 ± 5.1	42.0 ± 3.1	40.7 ± 1.6
CD8, %	23.3 ± 2.6	26.5 ± 1.3	28.8 ± 5.7
CD4:CD8	2.1 ± 0.3	1.6 ± 0.2	1.4 ± 0.2
Monocytes, %	18.6 ± 1.3	20.3 ± 2.1	17.4 ± 2.4
DR, %	19.1 ± 1.2	19.6 ± 7.2	17.4 ± 5.8

immunologic markers in the peripheral blood. As noted in Table 2, a significant increase was observed in the percentage of HLA–DR-bearing cells, monocytes, and CD8 (suppressor/cytotoxic) lymphocytes, and a significant decrease in the percentage of CD4 (helper/inducer) lymphocytes and the calculated CD4:CD8 ratio, after exposure to HBO ($P < 0.05$ in repeated measures ANOVA) (Table 2). No difference was found in absolute lymphocyte counts at the three time points (45.38 ± 2.8 vs. 46.88 ± 3.4 vs. 42.38 ± 2.3 × 10^6 cell · ml^{-1}, mean ± SE). No statistical difference was found in the percentage of B cells, total T lymphocytes (CD3), and natural killer (NK) cells immediately on leaving the hyperbaric chamber and 24 h later, compared to base-line values.

Figure 1 presents individual curves for the 13 subjects exposed to HBO, to demonstrate the consistent pattern of immunologic variation after hyperoxic exposure. As can be seen in Fig. 1*A*, immediately after one HBO session the CD8 lymphocyte level was elevated in each subject, whereas the individual CD4 level (Fig. 1*B*) decreased compared to base-line values. Twenty-four hours later, the percentage of CD4 lymphocytes continued to decline in some subjects, whereas in others the trend was a return to base-line values. On the whole, no significant difference was noted in CD4 mean values 24 h postexposure compared to base-line data. CD8 values were significantly higher compared to control values 24 h postexposure, and in most subjects they were even higher than or at least similar to the corresponding values immediately after HBO exposure. If we follow the individual traces of CD4:CD8 ratios (Fig. 1*C*), we can see that without exception each subject reached a considerably lower value on leaving the hyperbaric chamber, and even 24 h posthyperoxic exposure the CD4:CD8 ratios for some subjects were still below the normal range (1.5). Only some data were collected for an extended time (48 and 72 h after the HBO exposure). These data were not included in the statistical analysis but are presented in Fig. 1 together with the other individual curves. Twenty-four hours after HBO exposure, monocyte levels had returned to base-line values. HLA–DR-bearing cell values continued to increase ($P < 0.05$) for as long as 24 h posthyperoxic exposure. By way of comparison, relative changes in the parameters measured in our study are summarized in Fig. 2 and expressed by the percentage difference from control values.

Table 2: Blood Mononuclear Cell Populations in the Peripheral Blood of Healthy Subjects (n = 13) Exposed to 0.28 MPa Oxygen for 90 min (mean ± SE)[a]

	Base-line Control	On Exit From HBO Exposure	24 h Later
CD3, %	59.3 ± 3.1	57.2 ± 3.0	55.2 ± 2.2
CD4, %	47.5 ± 3.9	35.0 ± 3.2*	38.0 ± 2.2
CD8, %	20.0 ± 1.7	34.2 ± 2.1*	33.3 ± 3.7*
CD4:CD8	2.4 ± 0.2	1.0 ± 0.1*	1.2 ± 1.2*
Monocytes, %	18.3 ± 3.1	26.7 ± 2.0*	23.6 ± 2.5
DR, %	19.6 ± 1.9	34.1 ± 3.8*	39.1 ± 3.8*
B cells, %	13.5 ± 2.0	12.1 ± 2.1	17.5 ± 2.6
NK, %	8.5 ± 0.5	9.5 ± 1.4	10.1 ± 1.2

[a] *Asterisks* indicate significant differences ($P < 0.05$ in Wilcoxon's signed rank test)

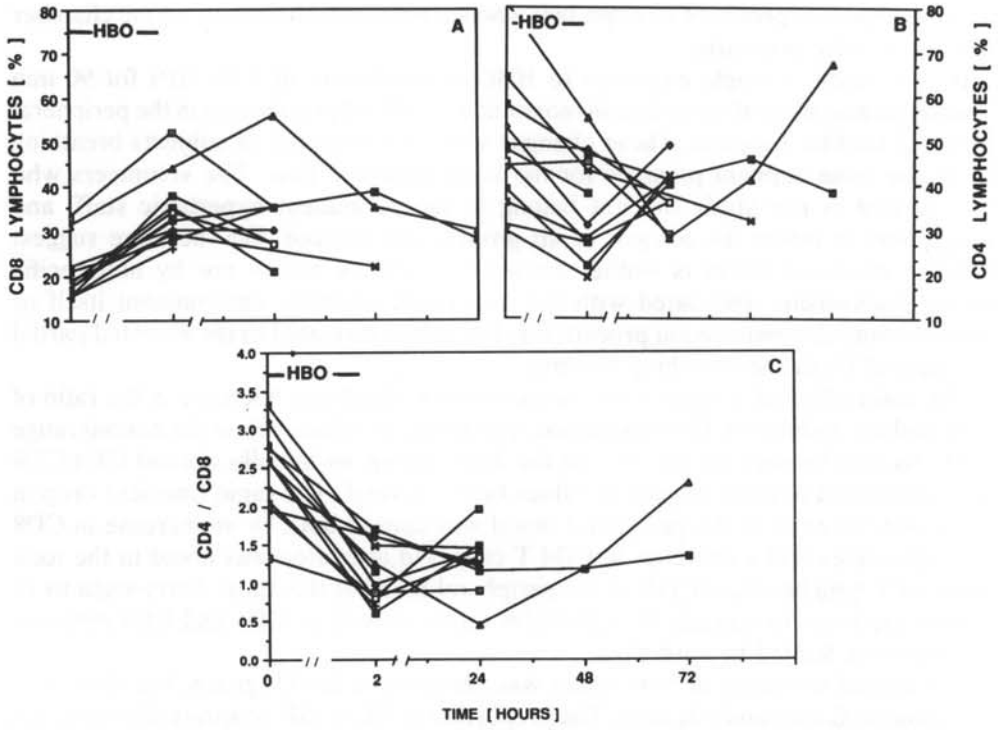

FIG. 1—Individual curves for the percentage of CD4, CD8, and the CD4:CD8 ratio in 13 healthy subjects exposed to HBO treatment: base-line data measurement taken on leaving the hyperbaric chamber (2 h) and 24 h later. *A*, percentage of CD8; *B*, percentage of CD4; *C*, CD4:CD8 ratio. *Note* the lack of continuity in the time scale.

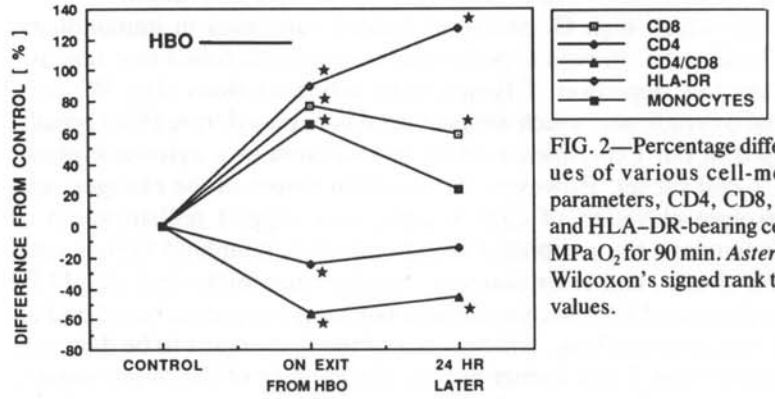

FIG. 2—Percentage difference from control values of various cell-mediated immunologic parameters, CD4, CD8, CD4:CD8, monocytes, and HLA–DR-bearing cells, on exposure to 0.28 MPa O_2 for 90 min. *Asterisks* indicate $P < 0.05$ in Wilcoxon's signed rank test compared to control values.

DISCUSSION

The main purpose of this study was to examine the effects of a single exposure to HBO on peripheral blood mononuclear cell populations in healthy human subjects. Because HBO is generally used at 0.25–0.28 MPa, we studied the effect of a commonly used hyperoxic therapeutic regimen (100% O_2 at 0.28 MPa for 90 min) and compared

it to the exposure profile of an attendant who accompanies the patient in the chamber (air at the same pressure).

In this study, a single exposure to HBO at a pressure of 0.28 MPa for 90 min resulted in a number of variations in mononuclear cell subpopulations in the peripheral blood of healthy subjects. These changes were not observed in subjects breathing air at the same ambient pressure for the same period of time. The volunteers who participated in our study did not belong to the permanent hyperbaric staff, and assignment to either the oxygen or air groups was random. We therefore suggest that the observed effect is neither caused by pressure per se nor by nonspecific stress mechanisms associated with the hyperbaric chamber environment itself or compression–decompression procedures, but rather is related to the elevated partial pressure of O_2 in the breathing mixture.

The main effect of a single HBO session was a significant decrease in the ratio of CD4 (helper, inducer):CD8 (suppressor, cytotoxic) to values below the normal range (1.5). As can be seen in Fig. 1*C*, in the HBO group an initially normal CD4:CD8 ratio decreased in each subject to values below normal. The rapid transient drop in the CD4:CD8 ratio in the peripheral blood was caused both by an increase in CD8 T lymphocytes and a decrease in CD4 T cells. No alteration was noted in the total count of T lymphocytes (CD3) in the peripheral blood at this time. Forty-eight to 72 h after exposure to oxygen the CD4:CD8 ratios as well as CD4 and CD8 percents and numbers tended to normalize.

A transient elevation of monocytes was observed in the O_2 group, but there was no change in B cells and NK cells. The proportion of HLA–DR-bearing cells increased significantly immediately after HBO exposure and continued to increase 24 h later. When activated, mononuclear cells in the peripheral blood can either elevate their constitutive HLA–DR surface concentration (i.e., B cells and monocytes) or express newly synthesized HLA–DR molecules (i.e., T cells). Although the identity of HLA–DR-bearing cells was not investigated in this study, it can be stated that elevation of these antigens reflects HBO-induced immunologic activation.

The mechanisms by which high O_2 pressures induce variations in immunologic competence are still unknown. In vivo experiments have demonstrated that reactive O_2 species cause selective depletion of lymphocyte subpopulations (16). We may therefore assume that oxyradicals, which are produced in excess during HBO breathing, may have caused in our experiments death or induction of a cytostatic phase to a more sensitive T-cell subset. However, the transient nature of the changes, and the relative and absolute elevation of CD8 T cells, may suggest redistribution of lymphocyte subsets between the peripheral blood and other lymphoid tissues such as the spleen, lymph glands, and bone marrow. Another possibility that should be considered is redistribution of lymphocyte subsets between peripheral blood and an injured target organ (possibly the lung, which is one of the first organs to be damaged by HBO). The mechanism of T-cell redistribution, the identity of the target organs, and the possible role of oxyradicals or other humoral mediators are not yet clear.

Further experiments in rats done in our laboratory have provided evidence of oxygen-induced redistribution of CD4 and CD8 T cells to the lungs and regional lymph nodes (manuscript in preparation).

It is known that numerous kinds of stress are associated with immunomodulation (17, 18). Typical examples are various types of physical exercise (19), transmeridian flight (20), and auditory stressors (21). It is interesting that although acute exposure

to a stressor can suppress a humoral immune response, repeated exposure results in apparent adaptation and in some cases an enhanced response (17, 21). The difference between a single exposure and repetitive exposures to a stressor has been studied intensively in relation to physical training and acute exercise challenge, demonstrating that the cell-mediated immune response to acute exercise is different from the response to chronic physical training, both in man (22) and in animals (23, 24). Moreover, the distribution of T-lymphocyte subpopulations in the peripheral blood after acute and chronic exercise stress was not found to reflect their distribution in different lymphoid compartments such as lymph nodes, thymus, and spleen (23).

Previous human studies conducted after 20 successive daily HBO treatments (12) failed to demonstrate any effect on the immune system of healthy subjects. We therefore suggest that the cell-mediated immune system (at least as expressed in blood mononuclear subsets) responds differently to "acute" (single) and "chronic" (long-term, repetitive) hyperoxic exposures. This might be of importance in designing protocols for HBO therapy, and particularly in determining the intervals between successive HBO sessions. For example, for better transplantation results, one HBO session might be considered before transplantation, thus carrying out the operation at the time of maximal decrease of CD4:CD8 ratio. A similar concept has been applied by Ritchie et al. (15), who suggested that to avoid allograft rejection, transplantation should be carried out at a specific hour of the day selected in accordance with a specific lymphocyte subset level.

At this stage, no definite conclusions can be drawn regarding the clinical implications of the acute variations in peripheral blood mononuclear populations found in our study to the overall immunologic competence of the healthy person. However, our findings suggest a unique advantage in employing a single exposure to HBO in appropriate clinical conditions in which an acute, reversible decrease in blood CD4:CD8 ratio may be beneficial. Another aspect of the problem which remains to be studied is the effect of a single HBO treatment on patients who already have a disturbed immunologic status due to infection, surgical and traumatic insults, or autoimmune diseases and immune deficiencies.

We thank Mr. Richard Lincoln for his assistance in the preparation of the manuscript. We thank all the volunteers from the INHI staff—physicians, technicians, and nurses—for taking part in this project.

The opinions presented herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Israeli Naval Hyperbaric Institute.—*Manuscript received October 1992; accepted March 1993.*

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