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Immune Function and Incidence of Infection during Basic Infantry Training

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The effect of an 18.5-week infantry training program on health status was studied in 23 male military personnel (aged 22.0 ± 0.5 years, mean ± SE). Aerobic power, body composition, and immune function (including natural killer cell activity, mitogen-stimulated lymphocyte proliferation, in vivo cell-mediated immunity, and secretory immunoglobulin A levels) were measured in subjects at the beginning and end of the course. Subjects self-reported their symptoms of sickness in health logs using a precoded checklist. Data from this study indicate that subjects became leaner and maintained, but did not increase, their aerobic fitness by the end of the course. Cell function was enhanced significantly; however, in vivo cell-mediated immunity remained the same, and levels of secretory immunoglobulin A were lower by the end of the course. The incidence of infection remained stable throughout the course. These results indicate that the current pattern of infantry training does not have an adverse effect on the health status of recruits.

Introduction

Military training programs may be very rigorous, involving not only prolonged periods of heavy physical activity but also exposure to psychological stressors, sleep deprivation, a negative energy balance, shifts of circadian rhythm, and exposure to extremes of hot and cold environments. The effects of such challenges on a soldier's health are complex, in part because of interactions between the various stressors. Anecdotal and documented reports[1,2] have suggested that military personnel attending lengthy and physically challenging training courses are predisposed to a decrement in immune function and an increase in susceptibility to infectious disease. From an occupational health perspective, immune suppression could impair both physical and mental performance.[3]

There is increasing evidence that severe physical and/or emotional stress can compromise the body's immune defenses. Controlled studies completed in our laboratory and elsewhere have demonstrated that a single bout of strenuous exercise, as well as periods of very heavy conditioning, can reduce certain aspects of immune function. Changes include a decrease in circulating natural killer (NK) cell count and cytolytic activity,[4] a decrease in mitogen-stimulated lymphocyte proliferation,[5,6] and a depressed cutaneous response to standard antigens.[7] The immune response to psychological stressors is more equivocal; increased NK cell activity[8] and a reduction or no change in mitogen-stimulated lymphocyte proliferation[9] have been reported.

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The purpose of this study was to investigate the health effects of participation in an infantry training course that was designed to prepare soldiers for basic duties within a platoon context. The course covers topics such as handling and firing infantry platoon and section weapons, communication procedures, physical tasks, offensive operations, and defensive operations and patrols. Soldiers undergo intensive instruction in a classroom setting as well as participate in regular physically demanding activity (including daily marches ranging from 5 to 10 km and regularly scheduled 5-km runs followed by push-ups, sit-ups, and chin-ups). On average, soldiers are involved in physical training 2 to 3 hours per day, 5 days per week. It was hypothesized that subjects would become immunosuppressed during the training program and, as a result, would suffer an increased incidence of upper respiratory infections and/or flu-like symptoms.

Materials and Methods

Twenty-three male subjects, enrolled in an 18.5-week Canadian qualification level regular infantryman training course (Meaford, Ontario; September 21, 1998, to February 10, 1999), completed the study. They ranged in age from 19 to 27 years (21.8 ± 0.5 years, mean ± SE). Subjects signed informed consent forms in accordance with a protocol approved by the Defence and Civil Institute of Environmental Medicine's human ethics committee.

At entry into and at the end of the training course, anthropometric measurements were made and percent body fat[10] was estimated. A submaximal cycle ergometer test was performed to predict each subject's maximal aerobic power (maximum oxygen consumption[11]) according to the method of Åstrand and Rodahl.[12]

Blood samples were collected at entry into the study as well as before and after a 13-km forced march (carrying a 20-kg backpack over hilly terrain), before and after a 2-week winter break (December 21, 1998 to January 4, 1999), and at the end of the course. Samples were obtained between 10:00 a.m. and 1:00 p.m. (except for the post-13-km-march sample, which was obtained at 4:00 p.m.). Complete blood counts were determined using an automatic hematology system (Coulter Electronics, Hialeah, Florida), and immunophenotyping was performed by flow cytometry.[13] Cell surface antigens were labeled with mouse anti-human monoclonal antibodies (mAbs; Becton-Dickinson, Mississauga, Ontario) conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), or peridinin chlorophyll protein in the following staining combinations: anti-CD45 mAb (FITC)/anti-CD14 mAb (PE), anti-CD3 mAb (FITC)/anti-CD19 mAb (PE), anti-CD4 mAb (FITC)/anti-CD8 mAb (PE)/anti-CD3 mAb (peridinin chlorophyll protein), and anti-CD3 mAb (FITC)/anti-CD16 mAb (PE)/

anti-CD56 mAb (PE). NK cell cytolytic activity was determined on isolated peripheral-blood mononuclear cells using a ^{51}Cr-release assay[13] and adjusted by the exponential curve-fitting method of Pross et al.[14] Phytohemagglutinin-stimulated lymphocyte proliferation was assessed on isolated peripheral-blood mononuclear cells.[15] Total serum cortisol concentrations were determined using a chemiluminescent enzyme immunoassay (Immulite, Diagnostic Products, Los Angeles, California). Cell counts and cortisol values, obtained after the 13-km forced march, were adjusted for any exercise-induced changes in blood or plasma volume, respectively, relative to the previous day's preexercise value using the method of Dill and Costill.[16]

Cell-mediated immunity was assessed at the beginning and end of the study using the CMI Multitest device (Connaught Laboratories, Toronto, Ontario), for two toxoids (tetanus and diphtheria), three bacterial antigens (streptococcus, tuberculin, and proteus), and two fungal antigens (candida and trichophyton). After 48 hours, all tested sites were measured; a positive reaction was recorded when a given site showed an induration of 2 mm or more in diameter compared with a negative control (glycerin).

Saliva samples were collected at the beginning and end of the study using a Salivette device (Sarstedt, St. Leonard, Quebec). The immunoglobulin A (IgA) concentration in each saliva sample was determined using a Radial Immuno-diffusion Binaird kit (The Binding Site, Birmingham, England).

Monthly logs for daily recording of health problems were provided with careful verbal and written instructions to each of the recruits. Subjects were required to record health problems on each day of the week using the codes reported previously by Nieman et al.[17]: (1) no health problem; (2) cold symptoms; (3) flu symptoms; (4) nausea, vomiting, and/or diarrhea; and (5) muscle, joint, or bone problems/injury. Subjects were also required to rate the severity of symptoms (mild, moderate, or severe). An episode of upper respiratory tract infection was identified when subjects coded for cold or flu symptoms for more than 2 days. Subjects were told how to distinguish colds from allergy by the presence or absence of colored discharge, respectively. Moreover, subjects were asked if they had allergies at entry into the study.

Results are expressed throughout as means ± SE. Descriptive statistics, including mean and SEM, were determined for each time point using Statview (SAS Institute, Cary, North Carolina). A one-way analysis of variance for repeated measures (six measures of time) was performed using SuperAnova (Abacus Concepts, Berkeley, California). Skin-test data were compared using a paired sign test for the cumulative indices and the size of individual indurations. A χ^2 test was also used to evaluate the percentage of positive responders for each individual antigen.

Results

Physical Characteristics

Subjects were of average height (1.75 ± 0.14 m), body mass (76.5 ± 1.8 kg), percent body fat (16.9 ± 0.6%), and level of aerobic physical fitness (predicted maximal oxygen intake of 43.4 ± 1.0 mL kg^{-1} min^{-1}). Participation in the course did not significantly alter body mass (final value, 76.3 ± 1.7 kg) or level of physical fitness (final predicted maximal intake, 41.6 ± 1.5 mL kg^{-1} min^{-1}). However, the percentage of body fat was reduced significantly by the end of the course (to 15.8 ± 0.5%, a change of 1.1%).

Immunological Data

The average leukocyte count for each subject decreased within the expected normal range of 4.5 to 11.0 × 10^9 cells/L[18] both at the beginning and at the end of the course (Fig. 1a). Total leukocyte counts were increased significantly compared with entry values both before and after the 13-km march but were similar to entry values by the end of the course. Granulocyte counts followed a similar pattern (Fig. 1b), being significantly increased both before and after the forced 13-km march. Overall, basic infantry training had no significant impact on granulocyte count. Lymphocyte counts (Fig. 1c) were not altered significantly by the end of the course, although there were slight, temporary decreases in lymphocyte numbers at the end of the 13-km march and before the winter break. Monocyte counts (Fig. 1d) did not change significantly during the course.

Course participation had little effect on circulating lymphocyte subsets (Fig. 2). T-, B-, and NK cell concentrations were similar at the beginning and end of the course. Transient changes within the lymphocyte subsets were observed in response to the 13-km forced march, including a significant reduction in the number of T and NK cells. Moreover, the number of T cytotoxic/suppressor cells was reduced before the 13-km march as well as before the winter break, and NK cell counts were increased after the winter break.

Both total NK cell activity (Fig. 3a) and the lymphocyte proliferative response (Fig. 3b) to phytohemagglutinin were significantly increased by the end of the course. Total NK cell activity followed a biphasic response, with a significant increase occurring before the winter break, a decrease to baseline levels after the break, and an increase again at the end of the course. In

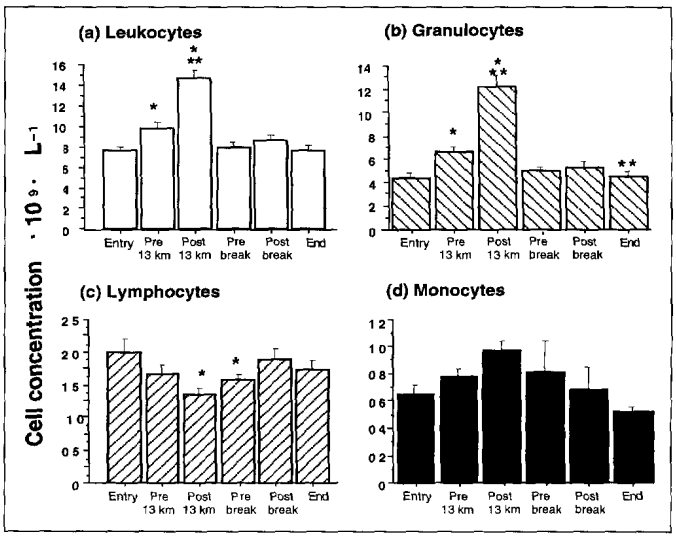

Fig. 1. Leukocyte and differential cell counts at the different time points. (a) Leukocyte count; (b) granulocyte count; (c) lymphocyte count; (d) monocyte count. One asterisk indicates a significant difference ($p < 0.05$) vs. entry data, two asterisks indicate a significant difference ($p < 0.05$) vs before the 13-km march

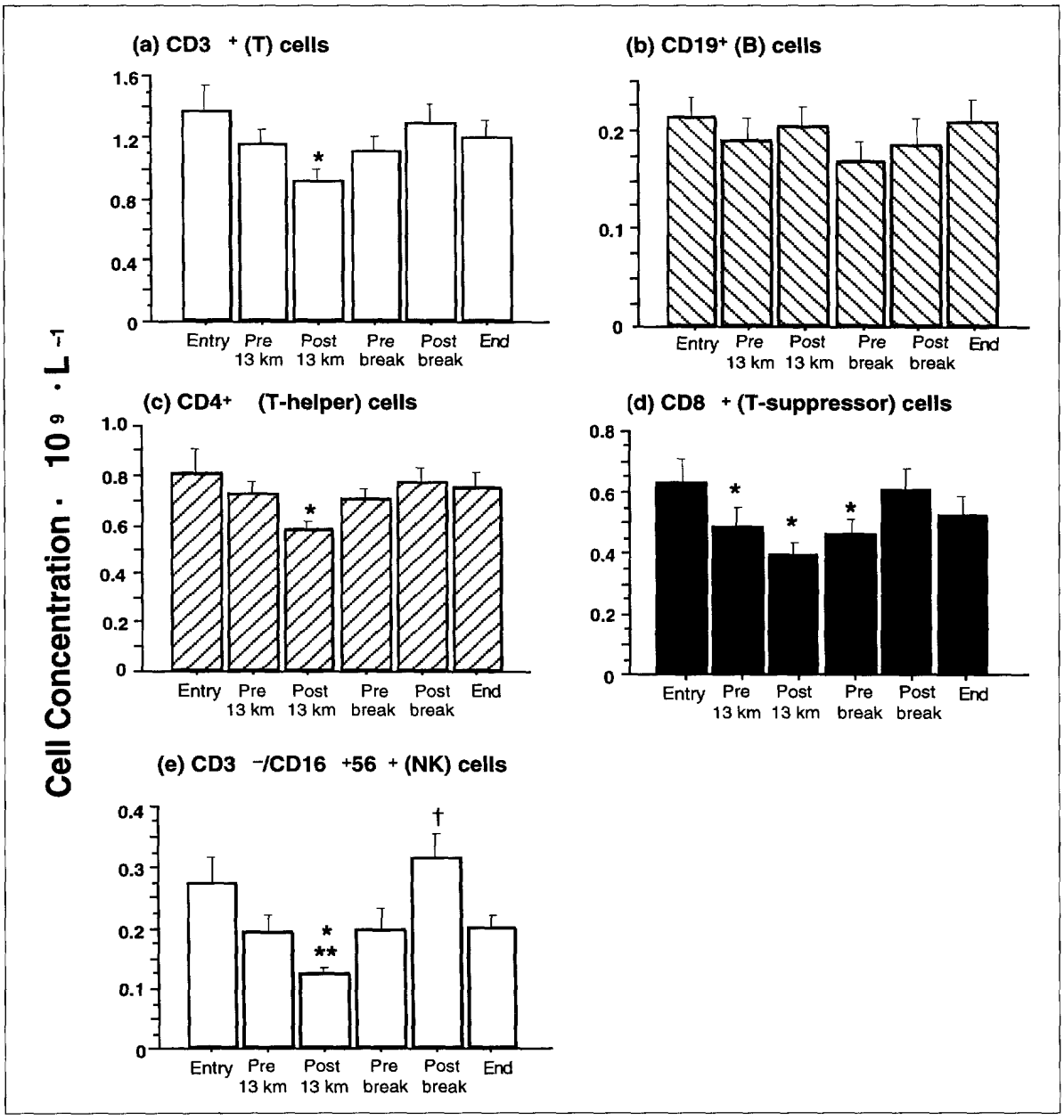

Fig. 2 Lymphocyte subset response to the different time points. (a) $CD3^+$ (T) cell response; (b) $CD19^-$ (B) cell counts, (c) $CD4^+$ (T-helper) cell; (d) $CD8^+$ (T-suppressor) cell counts; (e) $CD3^-/16,56^+$ (NK) cell counts. One asterisk indicates a significant difference ($p < 0.05$) vs entry data, two asterisks indicate a significant difference ($p < 0.05$) vs. before the 13-km march; a dagger indicates a significant difference ($p < 0.05$) vs. prebreak values.

contrast, the lymphocyte proliferative response tended to increase gradually throughout the course.

The mean pre- and post-training secretory IgA concentrations are presented in Figure 4. Values after training were significantly lower than at entry into the study. The cumulative responses (sum of the diameters of the indurations and the number of positive skin-test spots) for the CMI Multitest were similar at the beginning and end of training (Fig. 5a). The average induration size for each antigen was also relatively comparable between the beginning and the end of training (Fig. 5b). However, fewer individuals showed a positive reaction to the tetanus and proteus antigens by the end of the course (Fig. 5c).

Hormonal Response

Serum cortisol concentrations remained within the normal range of 82 to 635 nmol/L[18] throughout the study (Fig. 6), although the sample obtained before the 13-km march was increased significantly compared with the entry sample.

Health Problems

The incidence of colds remained relatively stable throughout the course. Twenty-two percent of subjects reported symptoms indicative of colds during the months of October, November, and January, and 28% reported such symptoms in December. Eleven percent of the subjects reported flu symptoms during the

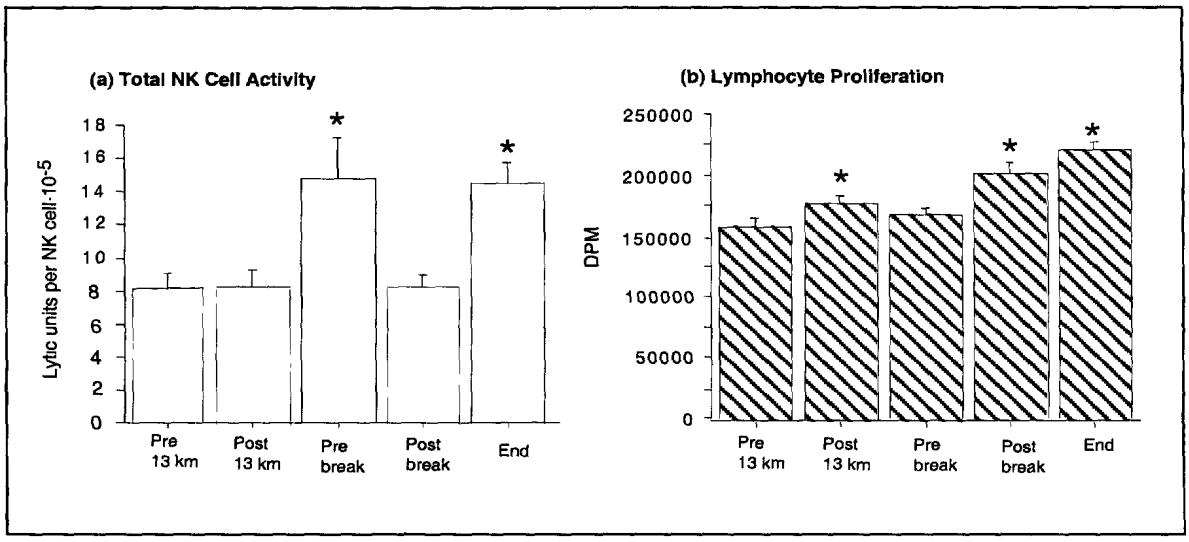

Fig. 3 Cell function at the different time points. (a) Total NK cell activity, (b) lymphocyte proliferation (phytohemagglutinin). An asterisk indicates a significant difference ($p < 0.05$) vs. before the 13-km march.

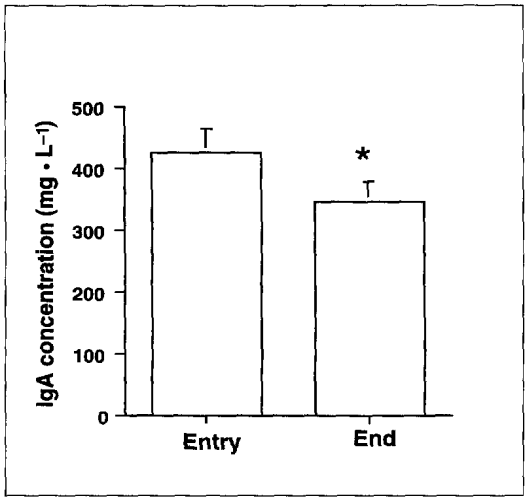

Fig. 4. Secretory IgA concentrations at the beginning and end of the course. An asterisk indicates a significant difference ($p < 0.05$) vs entry data.

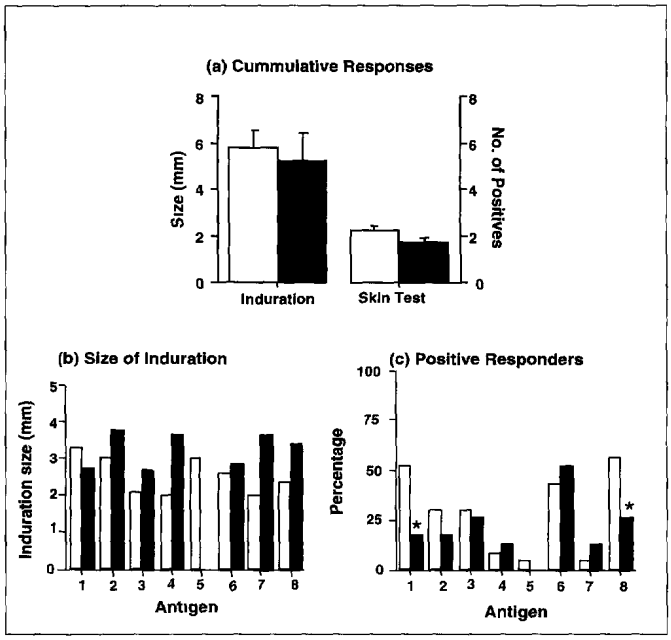

Fig. 5. The cell-mediated immunity (CMI) response at the beginning and end of the course White bars, entry data; dark bars, end data (a) Cumulative response (test score = average sum of positive induration diameters; number positive = average number of positive skin test areas); (b) average induration size at positive skin test sites; (c) percent of subjects responding positively to each antigen Antigen 1, tetanus; antigen 2, diphtheria; antigen 3, streptococcus, antigen 4, tuberculin; antigen 5, control; antigen 6, candida; antigen 7, trichophyton, antigen 8, proteus An asterisk indicates a significant difference ($p < 0.05$) vs. entry data.

months of December and January. Eleven percent of the subjects reported gastrointestinal problems in October, and 22% of the subjects experienced nausea and/or vomiting in January. However, most of the reported gastrointestinal disturbances appeared to be alcohol induced, because they occurred after holiday celebrations. Musculoskeletal problems were most frequent at the onset of the study; 28% of subjects sustained a musculoskeletal problem during the first month of training. Twenty-two percent of subjects reported musculoskeletal problems during the months of November and January, and only 5% reported problems during December. The low percentage of injuries occurring during December may be attributed to a decrease in training intensity and duration during that month.

Discussion

Overall, the design of the present field study had several strengths. It allowed for a simultaneous measurement of the health and immune status of a relatively large number of subjects. It also controlled for subject variability that would otherwise have been introduced by individual differences in the amount of sleep taken, nutrient intake, and activities of daily living. These factors are difficult to control in the usual laboratory experiment. Moreover, it is a direct representation of the type of training that military recruits must undergo during basic infantry training.

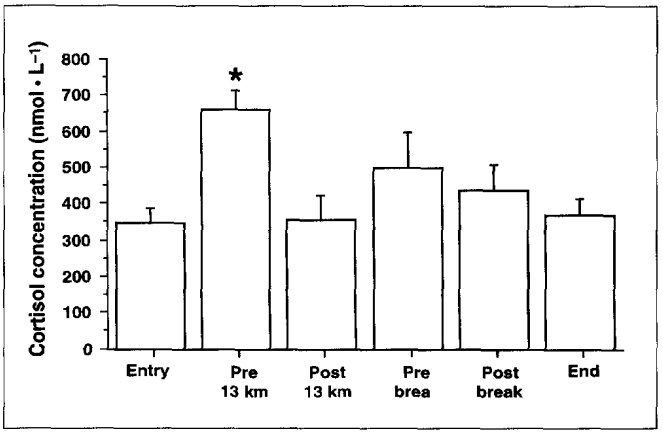

Fig 6. Serum cortisol concentrations at the different time points. An asterisk indicates a significant difference ($p < 0.05$) vs entry data.

Our findings of an increase in cell function (NK cell activity and lymphocyte proliferation), no change in cell-mediated immunity, and a relatively stable incidence of infection during the course do not support our initial hypothesis. It appears that the pattern of basic infantry training examined in this study does not compromise the soldier's health. In fact, participation had some beneficial effects, including an increase in lean body mass and maintenance of physical fitness.

These findings are in contrast with several reports that have associated very intense military training with either a decrement in immune function[1,19,20] or an increased susceptibility to infectious illness.[2,21] In vitro mitogen-stimulated lymphocyte proliferation was reduced in U.S. military personnel participating in basic cadet combat and Ranger training.[1,2,19,22] Linenger et al.[21] reported an increased incidence of respiratory infections in U.S. naval sea/air/land special warfare trainees participating in 25 weeks of particularly demanding training. They attributed this adverse outcome to the combined physical and psychological stresses of the training program. Likewise, Martinez-Lopez et al.[2] demonstrated that U.S. Army Ranger trainees became more susceptible to certain infectious illnesses (such as *Streptococcus pneumoniae* and group A β-hemolytic *Streptococcus pharyngitis*) as they progressed through three 2- to 3-week phases of very intense training.

Several reasons may account for why we did not observe the immunosuppression we had initially hypothesized. First, the physical component of basic infantry training is likely to have been much less than that experienced in the more specialized courses reported above. In support of this view, the soldiers' level of aerobic fitness remained unchanged throughout the course, suggesting that the main emphasis was on skill acquisition rather than physical conditioning. Second, the soldiers may have been under some stress at the beginning of our study and, as a result, we may not have obtained "true" resting values at entry to compare with data for the remaining time points. This criticism is certainly true for the data on NK cell function and proliferation, because we were unable to obtain data for these variables at the beginning of the training program. Finally, all of the subjects had previously undergone some basic combat training; this may have conditioned them to perceive infantry training as part of a routine rather than as a stressful experience.

Nieman[23] reported that physical conditioning increases NK cell activity. However, in the present study, subjects did not improve their level of physical fitness, and the increase in NK cell cytotoxicity that we observed before the winter break and at the end of the course may not have been a function of course participation. Other factors (such as anticipation of a break or completion of the training course) could have contributed to the observed increase in cell cytotoxicity.[8] Physical conditioning-induced changes in lymphocyte function have been reported inconsistently, and it is difficult to determine reasons for the progressive increase in lymphocyte proliferation that occurred throughout the study. Possibly, the soldiers were not in a resting state at entry into the study and the increase that we observed was a return toward baseline at the end of the course.

The delayed hypersensitivity response is an immunological reaction that occurs when foreign antigens are introduced into the skin. It involves a complex interplay between the accumulation of various leukocytes (T cells, NK cells, macrophages, and eosinophils) and the production of cytokines.[24] Because the cumulative response to the different antigens was unchanged at the end of the course, it can be assumed that the cellular response was not impaired. However, fewer subjects responded positively to the tetanus and proteus antigens upon completion of the course. The mechanism responsible for this antigen-specific discrepancy remains elusive.

Our findings that secretory IgA levels were reduced significantly by the end of the study initially appeared incongruent with our other findings. Secretory IgA protects mucosal surfaces and acts as an antiviral antibody.[24] Hence, the significant decrease in levels of secretory IgA should have been associated with an increased susceptibility to upper respiratory tract infections, but this did not occur. However, secretory immunoglobulin levels are increased in smokers,[25,26] and the reduction in secretory IgA levels that we observed may reflect the cessation of smoking that occurred among the soldiers. Forty-three percent of the soldiers were regular smokers at entry into the course, whereas all of them had voluntarily quit smoking by the end of the study.

The postexercise change in cell counts that we observed after the 13-km march is in agreement with other consistent reports.[23] Primarily hormonally mediated, the exercise-induced granulocytosis, lymphopenia, and changes in circulating lymphocyte subset counts were temporary, baseline values being restored within 24 hours. Because the changes were so short-lived and we did not observe a decrease in either in vitro or in vivo cell-mediated immunity, it is most unlikely that these subtle changes would have had an effect on the health of the soldiers.

Serum cortisol concentrations were analyzed to provide an index of the stress levels experienced by subjects as they progressed through the course. With the exception of the sample collected on the day before the 13-km march, values decreased within the expected normal range. Armario et al.[27] have shown that exposure to psychologically stressful situations can increase plasma cortisol concentrations. The 13-km march with a backpack is a battle fitness test that must be performed within 2 hours and 26 minutes; individuals who fail the test are removed from the course. Thus, the increased cortisol levels that we observed may reflect the psychological stress associated with anticipation of the test.

We conclude that basic infantry training (of the type examined in this study) does not have an adverse impact on either immune function or the incidence of upper respiratory infection. This probably reflects a relatively low intensity of physical stress throughout most of the course. However, the susceptibility to musculoskeletal injury at the beginning of the course requires further investigation. It is recommended that the basic infantry course be redesigned to include a physical conditioning component at the onset of the course that will improve rather than merely maintain the soldiers' level of aerobic fitness. An improved level of physical fitness would enable subjects to perform at the same performance level but with less stress to their system, thus reducing the risk of musculoskeletal injury.

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