Cold exposure: human immune responses and intracellular cytokine expression

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ABSTRACT

CASTELLANI, J. W., I. K. M. BRENNER, and S. G. RHIND. Cold exposure: human immune responses and intracellular cytokine expression. *Med. Sci. Sports Exerc.*, Vol. 34, No. 12, pp. 2013–2020, 2002. It is commonly believed that exposure to cold environmental temperatures depresses immune function and increases the risk for infection. This review paper will 1) present an overview of human physiological responses to cold exposure, 2) present the human studies examining the effects of cold exposure on immune responses, and 3) summarize recent experiments from our laboratories examining the effects of exercise and fatigue on immune responses during subsequent cold exposure. Based on the review of the literature, there is no support for the concept that cold exposure depresses immune function. **Key Words:** ANTARCTICA, EXERCISE, HYPOTHERMIA, NATURAL KILLER CELLS, SHIVERING

Many myths surround the idea that exposure to cold ambient temperatures causes human beings to contract colds or other respiratory tract infections caused by rhinoviruses. Indeed, use of the term "colds" may come from the common belief that cold exposure, in and of itself, causes upper respiratory tract infections (URTI). However, the data to support a link between cold ambient temperatures and reduced immune function and increased susceptibility to infections in humans is not well delineated. Two review papers have recently been published (55,56), with information focusing primarily on animal studies, which are difficult to extrapolate to humans. The purpose of this paper is to 1) present an overview of human physiological responses to cold exposure, 2) present the human studies examining the effects of cold exposure on immune responses, and 3) summarize recent experiments from our laboratories examining the effects of exercise on immune responses during subsequent cold exposure. The reader should be aware that changes in immune responses reported in this paper cannot be directly extrapolated to changes in host defense.

PHYSIOLOGICAL RESPONSES TO COLD EXPOSURE

Human beings rely primarily upon behavioral thermoregulation to maintain their body temperature during cold exposure (66). Humans wear clothing, seek shelter, and generate heat from various sources. However, there are many instances where these strategies are insufficient or unavailable. For example, individuals who engage in wintertime activities such as back-country skiing, snowshoeing, or military field-training exercises may not be dressed accordingly, may become wet and lose their clothing protection, may become fatigued and therefore cannot generate heat via exercise metabolism, or may not have access to heat-generating devices. In these cases, physiological adjustments must be made to maintain body core temperature. The two major physiological adjustments humans make when exposed to cold are 1) increased shivering thermogenesis and 2) increased peripheral vasoconstriction.

Humans increase metabolic heat production by initiating shivering activity. Shivering is an involuntary, repeated, rhythmic muscle contraction. Shivering contractions are the same as muscular contractions during exercise, except that no useful work is performed; thus, most of the contraction produces heat that aids in defending core temperature. It usually begins in the torso region and spreads to the limbs (8). Shivering is initially controlled by the rapid fall in skin temperatures and subsequently by the fall in body core temperature, with the core temperature accounting for ~67–80% of the thermoregulatory drive for shivering (24,57). Shivering can increase metabolic heat production 2–5 times above resting levels. For example, the oxygen uptake of men resting in 5°C air averaged 600–700 $mL \cdot min^{-1}$ (46). Cold-water exposure (18°C) elicits even greater falls in core temperature and higher oxygen uptakes (1 $L \cdot min^{-1}$) than cold-air exposure (67). The highest shivering rates observed were 2.2 $L \cdot min^{-1}$, which corresponded to ~ 46% $\dot{V}O_{2max}$ or a sevenfold increase in resting metabolism (31). Adult humans differ from smaller mammalian species (i.e., rats and mice) in that we have virtually no capability to increase metabolic heat production through nonshivering thermogenesis.

The other major mechanism for defending core temperature is to limit heat loss by peripheral vasoconstriction.

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Reducing peripheral blood flow reduces convective heat flow between the core and shell, increasing effective insulation (26). Vasoconstriction also causes skin temperature to fall thus decreasing the thermal gradient between the skin and external environment, effectively lowering heat transfer. Cutaneous vasoconstriction is maximal below skin temperatures of 31°C (59); further decreases in heat transfer occur by limiting blood flow to underlying muscle. Peripheral vasoconstriction redistributes blood to the core causing increased stroke volumes and small changes in heart rate (46); thus, cardiac output increases, which supports higher metabolic heat production.

The physiological adjustments to the cold, by themselves, can elicit immunological changes possibly not related to cold exposure, *per se*. For example, it is well known that exercise causes profound changes in the immune system (39,47) and because shivering contractions are similar to exercise, it could be that just exercise in general stimulates immune responses. Redistribution of blood flow, loss of plasma volume, and increases in cardiac output all have been observed to affect components of the immune system (54), and these physiological changes occur during both exercise and cold exposure.

One profound event that occurs during cold exposure is the activation of the sympathetic nervous system (SNS). Cold exposure causes plasma norepinephrine concentrations (a marker of SNS activity) to increase (15–17), and peripheral vasoconstriction is controlled by alpha-adrenergic receptors (22). Also, the SNS mediates and modulates immune function (54) and thus cold exposure can indirectly affect immune system responses through activation of the SNS. Definitive adrenergic blocking studies during cold exposure have not been done to confirm this hypothesis.

Plasma cortisol can modulate the immune system (54); however, the effects of cold exposure on plasma cortisol concentrations are equivocal (23,62,64). One problem with studies examining cortisol responses concerns pre- and post-cold exposure cortisol measurements occurring around late morning to mid-afternoon. Plasma cortisol levels normally fall during this time of the day (12) so it is difficult to discern if changes in plasma cortisol are due to the cold stress or to the normal diurnal rhythm. Another problem when analyzing cortisol changes is that this hormone is a steroid, so it is not synthesized rapidly after acute cold challenges, and therefore changes may not reflect its secretion and clearance rates.

Cold Exposure and Immune System: Previous Human Findings

Experimental (10,42) as well as anecdotal (36) reports have suggested that cold exposure increases susceptibility to infection (56). However, the majority of experimental data reporting blunted immune responses to cold exposure have been performed in mice and rats. The question is whether higher human infection rates are due to cold exposure *per se* as implied by the animal work, or due to other factors associated with cold exposure. Extrapolating data from animal studies for generalization to humans is also difficult. Many of the animal experiments, for example, used cold-water immersion as a stressor. Using this model, it is difficult to ascertain whether immune responses changed as a result of cold exposure or due to the fact that the animal was in a life-threatening situation with many other stressors present besides cold exposure.

Table 1 presents human studies on immune function after moderate cold exposure or over-wintering in Antarctica. Only a few of the studies employed controlled cold-exposure experiments and they were relatively brief in duration. The results have been equivocal with one study observing an increase in NK cell activity (41) and the other a decrease in cell proliferation after mitogen stimulation (38). Jansky et al. (37) found that acute cold-water immersion (14°C) increased white blood cell counts and that cold acclimation (6-wk of cold water immersion, 3 times per week) increased the percentage of $CD14^+$ and $CD25^+$ cells. From these limited studies, the data do not support the idea that cold exposure impairs immune responses that may be linked to overall function. Definitive tests to assess functionality need to be conducted.

Numerous studies have been performed in personnel stationed in Antarctica for long periods of time. The conclusion from these studies is that over-wintering in Antarctica does not appear to impact the immune system to the degree that infection risks are increased. It is unknown whether the degree of cold stress imposed on the personnel is great enough to cause changes in immune function. These personnel are well clothed and typically not outside in the severe cold weather for very long periods of time to incur significant cold strain. This type of cold exposure usually cools the face and hands but does not cause core cooling. The higher respiratory infection rates in the Antarctic summer are likely caused by exposure to people on supply ships who pass the virus onto the over-wintering population (56). These Antarctic studies are also difficult to use as a model of cold exposure because many other factors, including psychological ones, are common during isolation in Antarctica. Indeed, the National Aeronautic and Space Administration uses over-winterers in Antarctica as a model to examine isolation effects that may be present during space travel (43).

Perioperative hypothermia has been shown to affect immune responses. Only one study has directly examined the effect of perioperative hypothermia versus perioperative normothermia on the immune response. Briefly, Beilin et al. (7) observed, after surgery, that the group that became mildly hypothermic during surgery, had suppressed mitogenic, IL-1β, and IL-2 responses, compared with a group that remained normothermic. Whether these findings translate into higher infection rates after surgery where hypothermia is induced is debatable (6,40). Also, generalizing these results to nonsurgical patients exposed to cold is difficult. For example, the combination of cold and anesthesia may interact to decrease cell proliferation to mitogen stimulation, whereas mild hypothermia (34–35.5°C) induced with only cold exposure may potentially cause a

TABLE 1. Studies examining immune function in human subjects exposed to cold.

Study	Subjects	Exposures/Tests	Results
Cold-exposed ($> 35°C$ T_{core})			
Lackovic et al. (41)	8 men age = 20–26 yr	4°C air for 30 min (sitting nude)	T_{sl} ↓ by 0.45°C ↑ NE 3-fold ↑ NK cell activity
Jurankova et al. (38)	8 men age = 20–30 yr	4°C air for 30 min (sitting shorts)	↑ NE 3-fold optimal dose of PHA and PWM NC in proliferation sub-optimal dose of PHA ↓ proliferation 3–4 h post-cold
Delahanty et al. (18)	31 men age = 29.5 yrs (20–45) nonsmokers, no disease no hypertension	Cold pressor test Intermittent exposure of hand to 3°C water for 6 min	NC in NK cell activity NC in proliferation to PWM
Jansky et al. (37)	10 men age = 22 yr	60-min exposure to 14°C water acutely following 3x/wk–6 wk	Acute exposure - ↑ WBC count After 6 weeks - ↑ % of CD14+ and CD25+ cells ↑ TNF-α at rest
Antarctica studies			
Allen (4)	101 men, in Antarctica	Survey of cold symptoms	Long periods of isolation 0.1 colds/man/yr Summer relief periods 2.1 colds/man/yr
Allen et al. (5)	12 men, overwintering in Antarctica	Survey of cold symptoms Nasal secretions of subjects	8/12 have respiratory ailments Type of virus not identified
Williams et al. (63)	50 men, 2 women 33 in Antarctica (21–53 yr) 19 in sub-Antarctica (25–59)	CMI skin test	↑ induration size for sub-Antarctic group (24.9 mm vs 16.3 mm)
Warshauer et al. (61)	64 men finishing isolation 136 men just arrived in Antarctica	Incidence and severity of respiratory illness	39% of isolated group—colds 43%—newly arrived colds more prevalent in smaller living quarters
Mehta et al. (45)	14 men, 2 women (25–56 yr) in winter isolation	Saliva samples for EBV DNA shedding CMI skin test	↑ EBV DNA shedding CMI-varied response
Gleeson et al. (30)	65 men, 8 women age = 31 yr (23–55)	Salivary IgA, IgG, IgM over the course of a year	IgA, IgM low in first 4 months, return to baseline
Shearer et al. (53)	11 men in Antarctica age = 39 yr (24–55) 7 men in sub-Antarctica age = 36 (26–47)	Immunized subjects with T-cell dependent neoantigen NX-174 Measured viral clearance and primary and secondary Ab response	All cleared NX-174 in 7 d IgM to IgG switch No differences between groups
Perioperative hypothermia			
Beilin et al. (7)	30 hypothermic patients 30 normothermic patients age = 53 yrs	Mitogen proliferation Cytokine response	T_{es} 35.3°C in hypo and 36.3°C in normothermic Proliferation ↓ in hypothermic IL-1β, IL-2 ↓ in hypothermic NK activity ↓ in both groups
Roth-Isigkeit et al. (50)	14 patients undergoing coronary artery bypass graft (CABG) N = 7 (<50 yr old) N = 7 (>65 yr old)	IL-1β, IL-2, IL-6, IL-10, TNF-α before, during, and after CABG	No differences between age groups for any cytokine
Harig et al. (34)	30 patients undergoing CABG	IL-10 response during CABG	Hypothermia ↓ IL-10 (r = 0.80)
Hansen et al. (33)	21 patients undergoing eye surgery T_{core} ↓ to 32°C Hypotension (N = 14) induced with enalapril and nitroglycerin 7 control patients	IL-1β, IL-6, IL-10, TNF-α, adhesion molecules selection and ICAM-1	Hypotension had no effect on circulating cytokines ↑ ICAM-1 1-d postoperative (hypothermia ?)

T_{core}, core temperature; T_{sl}, sublingual; NE, norepinephrine; NK, natural killer cell; PWM, pokeweed mitogen; PHA, phytohemoagglutinin; NC, no change; CMI, cell-mediated immunity; EBV, Epstein-Barr virus; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; T_{es}, esophageal temperature; IL-1β, interleukin 1-beta; IL-2, interleukin 2; IL-10, interleukin 10; TNF-α, tumor necrosis factor; ICAM, intercellular adhesion molecule-1.

different response. For the most part, hypothermia studies are lacking, although Aibiki et al. (1) found elevated IL-6 and IL-8 levels upon hospital admission after accidental hypothermia to core temperatures $\leq 28°C$.

Immune responses during cold exposure with exertional fatigue. There are several populations (athletes and military personnel) that engage in high levels of activity and are exposed to cold. However, the interaction of exercise and cold exposure on immune function has not been well studied. Human data may be confounded by physical exertion being combined with cold exposure. For example, URTI are common in elite cross-country skiers (11), and studies of sustained military operations in the Canadian Arctic (52) have reported an increased incidence and severity of URTI. Are these changes due to cold exposure or to high levels of energy expenditure (or a combination of both)? It is established that intense exercise leads to transient decrements in immune function (47). Thus, these reports (11,52) do not support unequivocally that cold exposure suppresses immune function and increases the incidence of URTI. Also, cold exposure may also cause other physiological changes that are primarily responsible for affecting the immune response. Factors such as drying of the mucosal surface, a slowing of tracheal cilia (29) or a deterioration of the normal barrier function of the skin (32) may all impact immune responses and can be caused by prolonged cold exposure.

TABLE 2. Directional changes in cell counts, plasma IL-6, and norepinephrine during 120-min cold air exposure (5°C) after each of the 4 treatments; from Brenner et al.(13).

Treatment	Leukocytes	Granulocytes	Lymphocytes	Monocytes	NK cells	IL-6	NE
35°C-sit	↑	↑	↑	↔	↑	↑	↑
38°C-sit	↑	↑	↔	↑	↔	↑	↑
18°C-exercise	↑[a]	↑[a]	↔	↔[a]	↔	↑	↑
35°C-exercise	↑	↑	↔	↔	↑	↔	↔

NK, natural killer; IL-6, plasma interleukin-6; NE, plasma norepinephrine; Exerc., exercise; ↑, significant increase ($P < 0.05$) in variable; ↔, no change ($P > 0.05$) in variable
[a] Denotes significant difference ($P < 0.05$) between 18°C-Exerc. and 35°C-Sit. (60-min exposure at water temperature noted, exercise conducted at 55% $\dot{V}O_{2peak}$).

We have recently completed several studies examining the interaction of cold exposure and exercise. The experiments described below were performed in healthy humans who were well fed, maintained normal sleep patterns, and were not taking medication. The first study (13) examined cell changes and natural killer cell activity during acute cold-air exposure subsequent to an acute exercise bout. The second study (49) extended these findings and evaluated the interaction of cold and multiple days of strenuous exercise on intracellular cytokine expression and the immune system.

Study 1: Acute Exercise

This study examined immune responses in seven men during acute cold-air exposure (5°C, 2 h) after either rest or exercise in various water temperatures. With this experimental design, changes in immune function during cold exposure could be compared between prior exercise and no-exercise conditions, controlling for the normal rise in core temperature that accompanies exercise. The four experimental treatments were 1) passive exposure to 35°C water (no change in body core temperature), 2) passive exposure to 38°C water (increase core temperature ~ 1°C), 3) 60-min exercise (55% $\dot{V}O_{2peak}$) in 18°C water (no change in core temperature), and 4) 60-min exercise (55% $\dot{V}O_{2peak}$) in 35°C water (increase core temperature ~ 1°C).

Table 2 presents the directional results for cell counts and plasma hormones during the cold-air exposure after each of the four treatments. Cold-air exposure increased leukocyte and granulocyte counts regardless of whether the subjects exercised or had an elevated core temperature before cold exposure, whereas the lymphocyte, monocyte, and natural killer cell counts changes were more variable. Along with these changes in immune cell populations, plasma IL-6 and norepinephrine concentrations also increased during cold exposure. Interestingly, when cold exposure was preceded by exercise in 18°C water (no change in core temperature), the leukocyte, granulocyte, and monocyte cell counts were higher compared with the 35°C resting condition (also no change in core temperature). However, cell counts during cold exposure were independent of the initial core temperature, i.e., the immune responses between the two resting treatments or the two exercise treatments during subsequent cold exposure were not the same, even though the starting core temperatures were different before beginning cold exposure. To understand what physiological factors (heart rate, core temperature, and stress hormones) may be driving these changes in cell counts, a forward stepwise multiple-regression analysis was performed (Table 3). It was observed that heart rate, rectal temperature, and plasma stress hormones explained 2–42% of the total variance in total leukocytes, granulocytes, monocytes, and several lymphocyte subsets. Not surprisingly, norepinephrine accounted for most of the variance in these subsets. Norepinephrine can mobilize cells through adrenergic receptor stimulation (27) as well as by its action on sympathetic nerve terminals within the lymph nodes and spleen (9,14). The rise in circulating leukocytes during cold exposure may be attributed to a NE-mediated demargination of white blood cells (54). Demargination also may have occurred via the increase in cardiac output (and shear stress) that accompanies cold exposure.

To better understand immune function, versus changes in cell populations only, a ^{51}Cr uptake assay was performed to assess NK cell activity. Figure 1 indicates that NK cell activity during 5°C cold-air exposure increased after three out of four treatments (the exception was after exercise in 18°C water where NK cell activity was already 50–250%

TABLE 3. Multiple regression for prediction of leukocyte subset and lymphocyte counts, check marks indicate significance at $P < 0.05$; adapted from (13).

Variable	R^2	Heart Rate	T_{rectal}	Cortisol	Norepinephrine	Epinephrine
Leukocyte	0.45	√			√	
Granulocyte	0.40	√			√	
Monocyte	0.16		√			
Lymphocyte	0.27				√	
CD3+	0.20		√		√	
CD4+	0.02					
CD8+	0.22				√	
CD19+	0.42	√			√	√
Natural killer	0.19					

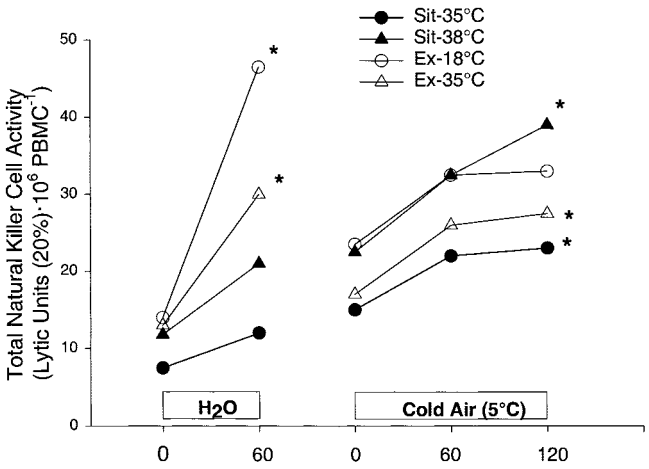

FIGURE 1—Total natural killer cell activity during each water pretreatment and during subsequent cold exposure. Redrawn from Brenner et al. (13). * Denotes significant increase ($P < 0.05$) in NK cell activity with respect to initial baseline measurement before water and cold exposure.

higher compared with the other treatments). Thus, it appears that one functional immune assay (NK cell activity) was not impaired after 2 h of cold-air exposure and suggests that the first-line defense against viral infections (NK cells) remains intact.

Study 2: Multiple Days of Exercise and Intracellular Cytokines

Cold exposure may initiate changes in cytokine expression associated with a nonspecific acute phase reaction (13,19,20). Cytokines play a key role in communicating between the neuroendocrine and immune systems (21) and therefore may influence immune homeostasis in cold environments (19,35). In humans, inflammatory cytokines may be released from circulating monocytes and include tumor necrosis factor (TNF)-α, followed by interleukin (IL)-1β, IL-6, and IL-1(ra) receptor antagonist (2,58).

To determine the interaction of cold exposure and exercise on cytokine expression in $CD14^+$ monocytes, nine men exercised in the cold on two occasions, on day 0, when the subjects were rested, and again on day 7, after 7 consecutive days of strenuous exercise (4 $h \cdot d^{-1}$). Cold exposure consisted of walking, completely wetted, for up to 6 h (from 1330 to 1930 h), in 5°C air at a wind velocity of 5.4 $m \cdot s^{-1}$. Blood samples were taken at 0700 h, after the 4-h exercise session (1130 h), and immediately after cold exposure (2000 h). Intracellular cytokine expression in $CD14^+$ cells was determined using flow cytometry. Intracellular expression of the cytokines is presented in Figure 2. $CD14^+$ monocytes were either stimulated without (LPS-) or with (LPS+) lipopolysaccharide, a monocyte specific antigen. The unstimulated response provides a "snap-shot" of what the cells are producing

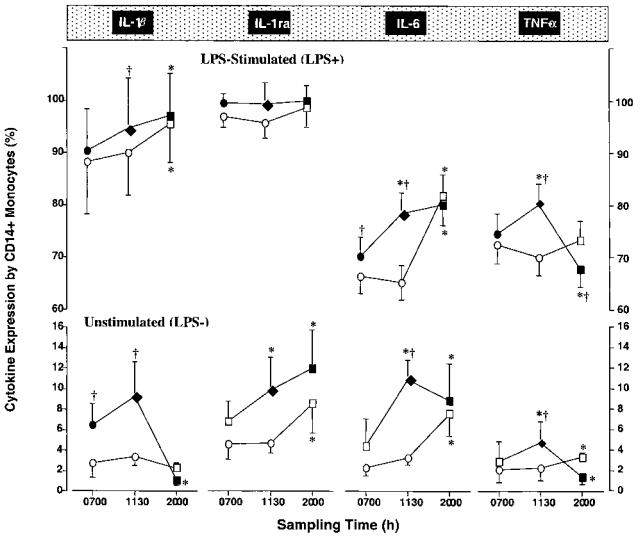

FIGURE 2—Percent cytokine expression (interleukin (IL)-1, IL-1ra, IL-6 and tumor necrosis factor (TNF)-α) by $CD14^+$ monocytes after unstimulated (LPS−) and stimulated (LPS+) treatment before exercise (0700; day 0, ○, day 7, ●), after exercise (1130; day 0, ○, day 7, ◆), and after cold exposure (2000; day 0, □; day 7, ■) before (day 0) and after 7 d (day 7) of exhaustive exercise. * Denotes significant ($P < 0.05$) within-trial differences vs resting (0700 h) values. † Denotes significant ($P < 0.05$) day 0 vs day 7 differences at times indicated; from Rhind et al. (49).

TABLE 4. Relationship between intracellular cytokines and catecholamines by stepwise multiple regression; adapted from (49).

Intracellular Cytokine	R^2	Probability Values	
		Norepinephrine	Epinephrine
IL-1β	0.39	0.02*	NS
IL-1ra	0.35	0.0001	0.0001
IL-6	0.34	0.006	0.02
TNF-α	0.40	0.01*	0.04*

* Negative association between variables.

in vivo at the time of sampling, whereas LPS stimulation gives an idea of the monocyte's capacity to respond when challenged. The two responses in concert reflect the overall ability of the monocyte to produce cytokines, which assesses the functionality of an immune cell to respond to a pathogen.

Figure 2 shows the intracellular cytokine results. IL-1β, in unstimulated $CD14^+$ monocytes, was elevated at rest and after exercise on day 7. Cold exposure on day 7, however, caused IL-1β expression to fall to levels observed after cold exposure on day 0. IL-6 expression in unstimulated cells was elevated after exercise on day 7. Cold exposure had no further effect on IL-6 expression after 7 d of exhaustive exercise, but on day 0, cold exposure increased intracellular IL-6 expression to levels observed on day 7. TNF-α expression, like IL-1β, was elevated with exercise in both stimulated and unstimulated cells, but cold exposure caused intracellular expression to significantly decline on day 7.

The mechanism underlying these changes in cytokine generation is not clear, but cytokine production may be related to induction of systemic endotoxemia, provoked by alterations in central hemodynamics and stress hormone release. Moderate cold exposure will reduce splanchnic blood flow (25), which promotes translocation of LPS into the systemic circulation (28). Also, upon re-warming the potential for even greater endotoxemia exists due to transient splanchnic reperfusion (25). Moreover, cold exposure may further exacerbate such effects via enhanced sympathoadrenal activation (51) and/or by directly augmenting the biological activity of LPS (44).

The molecular signaling pathways involved in thermal stress-induced cytokine alterations are unknown. The current findings (Table 4) are consistent with studies indicating that adrenergic/noradrenergic mechanisms are intimately involved in the regulation of cytokine production with various forms of physical stress (3). Monocytes express both α and β-adrenergic receptors and binding of epinephrine or norepinephrine can activate or inhibit different signal transduction pathways (48). The stimulation of β_2-adrenoceptors during stress attenuates excessive synthesis of pro-inflammatory cytokines (IL-1β and TNF-α) and elevates anti-inflammatory cytokines (IL-6, IL-1ra, and IL-10) via increased cAMP (48,65). However, activation of α_2-adrenoceptors is associated with reduced cAMP levels and enhances pro-inflammatory cytokine synthesis (60). In this context, the current observations showing that cold exposure elicited differential changes in IL-1β and TNF-α suggests that α-adrenergic mechanisms possibly prevail after cold stress on day 0. In contrast, cold exposure on day 7 decreased monocytic TNF-α and IL-1β but stimulated IL-1ra

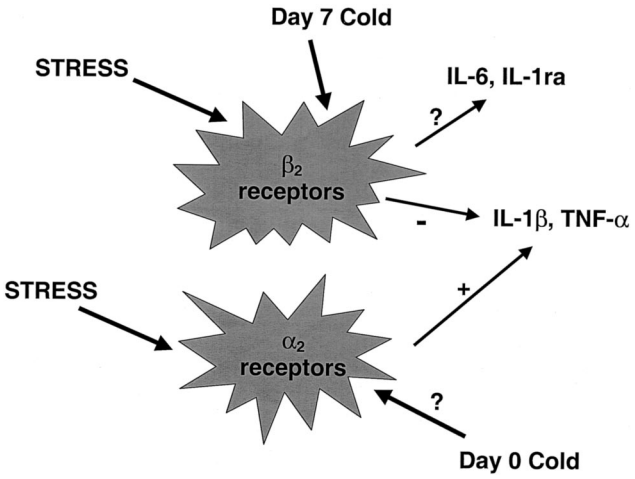

FIGURE 3—Proposed hypothesis for the effects of fatigue and cold on sympathetically mediated control of intracellular cytokine expression.

expression, indicating that β-adrenergic mechanisms may have predominated when cold stress was preceded by exercise. Alternatively, it is conceivable that repeated strenuous exercise for 7 d may alter monocytic adrenoceptor density (3), thereby reducing excessive synthesis of pro-inflammatory cytokines but enhancing anti-inflammatory cytokines after cold exposure. A schematic of this proposed hypothesis is presented in Figure 3.

We also examined the Th1-Th2 response to cold exposure. Th1 and Th2 refer to the T-helper cell type one and two responses, respectively, by $CD4^+$ cells when antigen-presenting cells such as macrophages present them with antigens. The Th1 response is associated with the release of the cytokines IL-2, gamma-interferon, and IL-12, which among other things, seem to promote the growth of cytotoxic, or killer, $CD8^+$ cells (cytotoxic T-cells). These cells, one of the main weapons of the cellular immune response, are critical in locating and killing infected cells. The Th2 response is associated with the release of IL-4, IL-5, and IL-10. These cytokines tend to encourage the production of antibodies. In addition to promoting antibodies, these cytokines actually suppress the Th1 response, reducing the magnitude of the cytotoxic T-cell response. The cytokines associated with Th1 in turn suppress the Th2 response. Our data (Rhind et al., unpublished observations) show that Th1 cytokines are down-regulated by cold exposure in the absence of a strong Th2 response, which suggests the possibility that cytotoxic $CD8^+$ cells will not be as effective after 3–6 h of cold exposure.

In conclusion, from the limited amount of data collected to this point, it appears that moderate acute cold exposure *per se* has no detrimental effect on the innate component of the immune system. It should be pointed out that these findings cannot be extrapolated directly to predicting host defense and susceptibility to pathogenic organisms. The mechanisms for these cold-induced natural killer cell and intracellular cytokine changes are still unknown at this point, although the literature suggests the sympathetic nervous system mediates many of the changes. Further studies using adrenergic-blocking drugs will clarify this. Also, because cold exposure affects cytokine production, cold exposure may provide a good physiological model to examine sympathetic nervous system regulation of cytokine production.

The views, opinions, and/or findings in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision unless so designated by other official designation. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USMRDC Regulation 70-25 on Use of Volunteers in Research.

REFERENCES

1. Aibiki, M., S. Maekawa, T. Nishiyama, K. Seki, and S. Yokono. Activated cytokine production in patients with accidental hypothermia. *Resuscitation* 41:263–268, 1999.
2. Akiyama, Y., G. W. Stevenson, E. Schlick, K. Matsushima, P. J. Miller, and H. C. Stevenson. Differential ability of human blood monocyte subsets to release various cytokines. *J. Leukoc. Biol.* 37:519–525, 1985.
3. Alkharfy, K. M., J. A. Kellum, and G. R. Matzke. Unintended immunomodulation: part II. Effects of pharmacological agents on cytokine activity. *Shock* 13:346–360, 2000.
4. Allen, T. R. Common colds in Antarctica. *J. Hyg.* 71:649–656, 1973.
5. Allen, T. R., A. F. Bradburne, E. J. Stott, C. S. Goodwin, and D. A. J. Tyrrell. An outbreak of common colds at an Antarctic base after seventeen weeks of complete isolation. *J. Hyg.* 71:657–667, 1973.
6. Beal, M. W., D. C. Brown, and F. S. Shofer. The effects of perioperative hypothermia and the duration of anesthesia on postoperative wound infection rate in clean wounds: a retrospective study. *Vet. Surg.* 29:123–127, 2000.
7. Beilin, B., Y. Shavit, J. Razumovsky, Y. Wolloch, A. Zeidel, and H. Bessler. Effects of mild perioperative hypothermia on cellular immune responses. *Anesthesiology* 89:1133–1140, 1998.
8. Bell, D.G., P. Tikuisis, and I. Jacobs. Relative intensity of muscular contraction during shivering. *J. Appl. Physiol.* 72:2336–2342, 1992.
9. Bellinger, D. L., K. D. Ackerman, S. Y. Felten, D. Lorton, and D. L. Felten. Noradrenergic sympathetic innervation of thymus, spleen and lymph nodes: aspects of development, aging and plasticity in neural immune interaction. In: *Interactions among CNS, Neuroendocrine and Immune Systems*, J. W. Hadden, K. Masek, and G. Nistico (Eds.). Rome: Pythagora, 1989, pp. 35–66.
10. Ben-Nathan, D., S. Lustig, and D. Kobiler. Cold stress-induced neuroinvasiveness of attenuated arboviruses is not solely mediated by corticosterone. *Arch. Virol.* 141:1221–1229, 1996.
11. Berglund, B., and P. Hemmingsson. Infectious disease in elite cross-country skiers: a one-year incidence study. *Clin. Sports Med.* 2:19–23, 1990.
12. Brandenberger, G. Cortisol responses to exercise and their interactions with diurnal secretory peaks. In: *Exercise Endocrinology*, K. Fotherby and S. B. Pal (Eds.). Berlin: de Gruyter, 1985, pp. 47–64.
13. Brenner, I. K. M., J. W. Castellani, C. Gabaree, et al. Immune changes in humans during cold exposure: effects of prior heating and exercise. *J. Appl. Physiol.* 87:699–710, 1999.
14. Bulloch, K., and T. Radojcic. Characterization of muscarinic acetyl choline and beta-adrenergic receptors on fresh and cloned immunocytes. In: *Interactions among CNS, Neuroendocrine and Immune Systems*, J. W. Hadden, K. Masek, and G. Nistico (Eds.). Rome: Pythagora, 1989, pp. 17–34.
15. Castellani, J. W., A. J. Young, D. W. Degroot, et al. Thermoregulation during cold exposure after several days of exhaustive exercise. *J. Appl. Physiol.* 90:939–946, 2001.
16. Castellani, J. W., A. J. Young, J. E. Kain, A. Rouse, and M. N. Sawka. Thermoregulation during cold exposure: effects of prior exercise. *J. Appl. Physiol.* 87:247–252, 1999.

17. CASTELLANI, J. W., A. J. YOUNG, M. N. SAWKA, and K. B. PANDOLF. Human thermoregulatory responses during serial cold-water immersions. *J. Appl. Physiol.* 85:204–209, 1998.
18. DELAHANTY, D. L., A. L. DOUGALL, L. HAWKEN, et al. Time course of natural killer cell activity and lymphocyte proliferation in response to two acute stressors in healthy men. *Health Psychol.* 15:48–55, 1996.
19. DUGUE, B., and E. LEPPANEN. Adaptation related to cytokines in man: effects of regular swimming in ice-cold water. *Clin. Physiol.* 20:114–121, 2000.
20. FAIRCHILD, K. D., R. M. VISCARDI, L. HESTER, I. S. SINGH, and J. D. HASDAY. Effects of hypothermia and hyperthermia on cytokine production by cultured human mononuclear phagocytes from adults and newborns. *JAMA* 20:1049–1055, 2000.
21. FELTEN, S. Y., K. S. MADDEN, D. L. BELLINGER, B. KRUSZEWSKA, J. A. MOYNIHAN, and D. L. FELTEN. The role of the sympathetic nervous system in the modulation of immune responses. *Adv. Pharmacol.* 42:583–587, 1998.
22. FRANK, S. M., N. EL-GAMAL, S. N. RAJA, and P. K. WU. Alpha-adrenoceptor mechanisms of thermoregulation during cold challenge in humans. *Clin. Sci.* 91:627–631, 1996.
23. FRANK, S. M., M. S. HIGGINS, L. A. FLEISHER, J. V. SITZMANN, H. RAFF, and M. J. BRESLOW. Adrenergic, respiratory, and cardiovascular effects of core cooling in humans. *Am. J. Physiol.* 272:R557–R562, 1997.
24. FRANK, S. M., S. N. RAJA, C. F. BULCAO, and D. S. GOLDSTEIN. Relative contribution of core and cutaneous temperatures to thermal comfort and autonomic responses in humans. *J. Appl. Physiol.* 86:1588–1593, 1999.
25. GAFFIN, S. L., F. B. DIETZ, J. G. BROCK-UTNE, et al. Rewarming from hypothermia leads to elevated plasma lipopolysaccharide concentrations. *Undersea Hyperbar. Med.* 27:1–7, 2000.
26. GAGGE, A. P., and R. R. GONZALEZ. Mechanisms of heat exchange: biophysics and physiology. In: *Handbook of Physiology: Environmental Physiology*. Bethesda, MD: American Physiological Society, 1996, pp. 45–84.
27. GANNON, G. A., P. N. SHEK, and R. J. SHEPHARD. Natural killer cells: modulation by intensity and duration of exercise. *Exerc. Immunol. Rev.* 1:26–48, 1995.
28. GERCEKOGLU, H., O. TARIM, I. AGAR, et al. Effects of hypothermia on blood endogenous endotoxin levels during cardiopulmonary bypass. *J. Card. Surg.* 12:223–227, 1997.
29. GIESBRECHT, G. G. The respiratory system in a cold environment. *Aviat. Space Environ. Med.* 66:890–902, 1995.
30. GLEESON, M., J. L. FRANCIS, D. J. LUGG, et al. One year in Antarctica: mucosal immunity at three Australian stations. *Immunol. Cell Biol.* 78:616–622, 2000.
31. GOLDEN, F. S. C., I. F. G. HAMPTON, G. R. HERVERY, and A. V. KNIBBS. Shivering intensity in humans during immersion in cold water. *J. Physiol. (Lond.)* 277:48, 1979.
32. HALKIER-SORENSEN, L., G. K. MENON, P. M. ELIAS, K. THESTRUP-PEDERSEN, and K. R. FEINGOLD. Cutaneous barrier function after cold exposure in hairless mice: a model to demonstrate how cold interferes with barrier homeostasis among workers in the fish-processing industry. *Br. J. Dermatol.* 132:391–401, 1995.
33. HANSEN, D., S. BOGATZKI, R. SYBEN, et al. Effect of profound normovolemic hypotension and moderate hypothermia on circulating cytokines and adhesion molecules. *Shock* 12:335–339, 1999.
34. HARIG, F., R. CESNJEVAR, F. O. MAHMOUD, and J. VON DER EMDE. Perioperative factors influencing interleukin-10 release under cardiopulmonary bypass. *Thorac. Cardiovasc. Surg.* 47:361–368, 1999.
35. HIMMS-HAGEN J. Neural and hormonal responses to prolonged cold exposure. In: *Handbook of Physiology, Environmental Physiology*. Bethesda, MD: American Physiological Society, 1996, pp. 439–480.
36. IYUN, B. F., and G. TOMSON. Acute respiratory infections-mothers' perceptions of etiology and treatment in south-western Nigeria. *Soc. Sci. Med.* 42:437–445, 1996.
37. JANSKY, L., D. POSPISILOVA, S. HONZOVA, et al. Immune system of cold-exposed and cold-adapted humans. *Eur. J. Appl. Physiol.* 72:445–450, 1996.
38. JURANKOVA, E., D. JEZOVA, and M. VIGAS. Central stimulation of hormone release and the proliferative response of lymphocytes in humans. *Mol. Chem. Neuropathol.* 25:213–223, 1995.
39. KEAST, D., K. CAMERON, and A. R. MORTON. Exercise and the immune response. *Sports Med.* 5:248–267, 1988.
40. KURZ, A., D. I. SESSLER, and T. K. HUNT. Perioperative normothermia to reduce the incidence of surgical wound infection and shorten hospitalization. *N. Engl. J. Med.* 334:1209–1215, 1996.
41. LACKOVIC, V., L. BORECKY, M. VIGAS, and J. ROVENSKY. Activation of NK cells in subjects exposed to mild hyper- or hypothermic load. *J. Interferon Res.* 8:393–402, 1988.
42. LEVINSON, S. O., A. MILZER, and P. LEWIN. Effect of fatigue, chilling and mechanical trauma on resistance to experimental poliomyelitis. *Am. J. Hyg.* 42:204–213, 1945.
43. LUGG, D. J., and M. SHEPANEK. Space analogue studies in Antarctica. *Acta Astronaut* 44:693–699, 1999.
44. LUHM, J., A. B. SCHROMM, U. SEYDEL, et al. Hypothermia enhances the biological activity of lipopolysaccharides by altering its fluidity state. *Eur. J. Biochem.* 256:325–333, 1998.
45. MEHTA, S. K., D. L. PIERSON, H. COOLEY, R. DUBOW, and D. J. LUGG. Epstein-Barr virus reactivation associated with diminished cell-mediated immunity in antarctic expeditioners. *J. Med. Virol.* 61:235–240, 2000.
46. MUZA, S. R., A. J. YOUNG, J. E. BOGART, and K. B. PANDOLF. Respiratory and cardiovascular responses to cold stress following repeated cold water immersion. *Undersea Biomed. Res.* 15:165–178, 1988.
47. PEDERSEN, B. K., and L. HOFFMAN-GOETZ. Exercise and the immune system: regulation, integration, and adaptation. *Physiol. Rev.* 80: 1055–1081, 2000.
48. PLATZER, C., W. DOCKE, H. VOLK, and S. PROSCH. Catecholamines trigger IL-10 release in acute systemic stress reaction by direct stimulation of its promoter/enhancer activity in monocytic cells. *J. Neuroimmunol.* 105:31–38, 2000.
49. RHIND, S. G., J. W. CASTELLANI, I. K. M. BRENNER, et al. Intracellular monocyte and serum cytokine expression is modulated by exhausting exercise and cold exposure. *Am. J. Physiol.* 281:R66–R75, 2001.
50. ROTH-ISIGKEIT, A. K., J. SCHWARZENBERGER, T. VAN BORSTEL, et al. Perioperative cytokine release during coronary artery bypass grafting in patients of different ages. *Clin. Exp. Immunol.* 114:26–32, 1998.
51. RUSSELL, J. A. Catecholamines and the splanchnic circulation. *Crit. Care Med.* 27:242–243, 1999.
52. SABISTON, B. H., and S. D. LIVINGSTONE. *Investigation of Health Problems Related to Canadian Northern Military Operations.* Toronto, ON: Defence and Civil Inst. Environ. Med., 1973.
53. SHEARER, W. T., D. J. LUGG, H. M. ROSENBLATT, et al. Antibody responses to bacteriophage ϕX-174 in human subjects exposed to the antarctic winter-over model of spaceflight. *J. Allergy Clin. Immunol.* 107:160–164, 2001.
54. SHEPHARD, R. J. Physical activity, training and the immune response. Carmel, IN: Cooper Publishing Group, 1997, pp. 8–9.
55. SHEPHARD, R. J., J. W. CASTELLANI, and P. N. SHEK. Immune deficits induced by strenuous exertion under adverse environmental conditions: manifestations and countermeasures. *Crit. Rev. Immunol.* 18:545–568, 1998.
56. SHEPHARD, R. J., and P. N. SHEK. Cold exposure and immune function. *Can. J. Physiol. Pharmacol.* 76:828–836, 1998.
57. TONER, M. M., and W. D. MCARDLE. Human thermoregulatory responses to acute cold stress with special reference to water immersion. In: *Handbook of Physiology: Environmental Physiology*. Bethesda, MD: American Physiological Society, 1996, pp. 379–418.
58. VAN FURTH, R. Human monocytes and cytokines. *Res. Immunol.* 149:719–720, 1998.
59. VEICSTEINAS, A., G. FERRETTI, and D. W. RENNIE. Superficial shell insulation in resting and exercising men in cold water. *J. Appl. Physiol.* 52:1557–1564, 1982.
60. VIZI, E. S. Receptor-mediated local fine-tuning by noradrenergic innervation of neuroendocrine and immune systems. *Ann. N.Y. Acad. Sci.* 851:388–396, 1998.
61. WARSHAUER, D. M., E. C. DICK, A. D. MANDEL, T. C. FLYNN, and R. S. JERDE. Rhinovirus infections in an isolated antarctic station:

transmission of the viruses and susceptibility of the population. *Am. J. Epidemiol.* 129:319–340, 1989.
62. WILKERSON, J. E., P. B. RAVEN, N. W. BOLDUAN, and S. M. HORVATH. Adaptation in man's adrenal function in response to acute cold stress. *J. Appl. Physiol.* 36:183–189, 1974.
63. WILLIAMS, D. L., A. CLIMIE, H. K. MULLER, and D. J. LUGG. Cell-mediated immunity in healthy adults in Antarctica and the sub-Antarctic. *J. Clin. Lab Immunol.* 20:43–49, 1986.
64. WITTERT, G. A., H. K. OR, J. H. LIVESEY, A. M. RICHARDS, R.A. DONALD, and E. A. ESPINER. Vasopressin, corticotrophin-releasing factor, and pituitary adrenal responses to acute cold stress in normal humans. *J. Clin. Endocrinol. Metab.* 75:750–755, 1992.
65. YOSHIMURA, T., C. KURITA, T. NAGAO, et al. Inhibition of tumor necrosis factor-α and interleukin-1β production by β-adrenoceptor agonists from lipolysaccharide-stimulated human peripheral mononuclear cells. *Pharmacology* 54:144–152, 1997.
66. YOUNG, A. J., J. W. CASTELLANI, and M. N. SAWKA. Human physiological responses to cold exposure. In: *The 1997 Nagano Symposium on Sports Sciences*, H. Nose, T. Morimoto, and E. R. Nadel (Eds.). Carmel, IN: Cooper Publishing Group, 1998, pp. 273–286.
67. YOUNG, A. J., S. R. MUZA, M. N. SAWKA, R. R. GONZALEZ, and K. B. PANDOLF. Human thermoregulatory responses to cold air are altered by repeated cold water immersion. *J. Appl. Physiol.* 60: 1542–1548, 1986.