Human Natural Killer Cell Subsets and Acute Exercise: A Brief Review

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ABSTRACT

Natural killer (NK) cells are the most responsive immune cell to acute exercise. This sensitivity to physiological stress combined with their role in innate immune defences suggests that these cells may be a link between regular physical activity and overall health status. NK cells are a heterogeneous population consisting of at least two distinct subsets based on the expression intensity of CD56. $CD56^{bright}$ and $CD56^{dim}$ cells exhibit different phenotypical and functional characteristics. In this review, we examine the effects of acute exercise on NK cell subsets, with special reference to potential health implications of the findings. The available evidence suggests a differential mobilization of NK cell subsets in response to acute exercise; $CD56^{bright}$ NK cells are less responsive than their $CD56^{dim}$ counterparts. During the post-exercise recovery period (up to 1h), the ratio of $CD56^{bright}$:$CD56^{dim}$ cells favours the $CD56^{bright}$ subset. The potential significance of these findings is discussed in the context of normal physiological adaptation to exercise. We also discuss the potential role of exercise in certain clinical conditions (e.g., multiple sclerosis) as an adjunct therapy to mobilize the $CD56^{bright}$ subset. Further investigation into the biology of NK cell subsets and exercise should prove to be a fruitful area for years to come.

Keywords: $CD56^{bright,}$ $CD56^{dim}$, acute exercise, humans, health, NK cells, NK subsets

INTRODUCTION

Physical *inactivity* is considered to be an independent risk factor for various chronic diseases of adulthood (3). While the mechanisms that translate a physically-active lifestyle into good health continue to be revealed, involvement of the immune system has received considerable attention in recent years. Indeed, the

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acute and chronic effects of exercise on numerous aspects of the human immune system are the focus of substantial research, but the health significance of this work remains to be unraveled. One aspect of the immune system that has garnished persistent interest is natural killer (NK) cells. The striking sensitivity of NK cells to exercise stress provides strong support that these cells may be implicated as a potential link between regular physical activity and overall health status. NK cells are a heterogeneous population of lymphocytes, the biology of which is under intense scrutiny given the clinical significance of NK cells in antiviral (2) and anti-cancer (5) defenses. New insights into the origin, development, and interaction of these cells with other immune factors and non-immune tissue represents an exciting and rapidly developing area of research and provides a framework to explore the significance of exercise-induced changes in these cell populations. Recent attention on NK cells has been driven by the presence of distinct NK cell subsets, which appear to hold diverse functions (see refs 7 and 9 for reviews).

In this brief review, we shall consolidate the literature on NK cell subsets and *acute* exercise in humans, while focusing on the heterogeneity of these cells. This is not a comprehensive review of exercise and NK cells (these are available elsewhere, e.g., (36)). Rather, the primary objective of this paper is to explore the effects of acute exercise on NK cell subsets (i.e., $CD56^{bright}$ and $CD56^{dim}$) with special reference to potential health implications of the findings. To achieve this objective, relevant published articles were retrieved by a PubMed database search using the following keywords: "exercise" *AND* "CD56". The reference lists of relevant articles identified through the PubMed search were then hand-searched for additional studies. In a few instances, data available in abstract form were included because this was the only source of relevant information. Studies that measured NK subsets in peripheral blood collected at a minimum before *and* immediately after an acute bout of exercise were chosen.

Human NK Cell Subsets – An Overview

NK cells are large granular lymphocytes with natural cytotoxicity (9). NK cells represent one component of innate immunity that can destroy certain virally-infected and tumour cells, without prior sensitization (i.e., non MHC-restricted). The widely accepted CD classification of NK cells includes the co-expression of the Fcγ receptor III (CD16) and an isoform of the human neural cell adhesion molecule (CD56) whose function on NK cells is unknown (9). The traditional phenotype of human circulating NK cells therefore has been: $CD3^{-}CD16^{+}CD56^{+}$. More than 20 years ago the existence of two unique and functionally different NK cell populations, based on the expression intensity of CD56 (Figure 1), was noted (23).

$CD3^{-}CD56^{dim}$ cells, which express high levels of CD16, are more cytotoxic than $CD3^{-}CD56^{bright}$ cells, which express low or no levels of CD16 (23). Mounting evidence suggests that the $CD56^{bright}$ subset, which comprises ~10% of circulating NK cells and possess the capacity to produce abundant cytokines (9), may be of particular relevance in the early events of immune challenge by coordinating "cross-talk" between innate and adaptive arms of immunity (13).

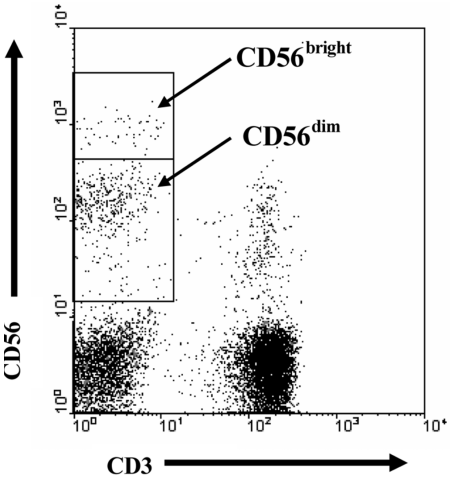

Figure 1. Flow cytometry characterization of NK cell subsets. $CD56^{bright}$ and $CD56^{dim}$ cell populations are derived from lymphocyte events gated based on forward- vs sidescatter characteristics.

Further phenotypical and functional differences between $CD56^{bright}$ and $CD56^{dim}$ cells are comprehensively contrasted in a recent review (7). A potentially important distinction between these subsets is the expression level of adhesion molecules. For example, $CD56^{bright}$ cells feature quite high levels of CD62L and it is believed that enhanced expression of these adhesion molecules on $CD56^{bright}$ cells favours their trafficking to lymph nodes and sites of inflammation, where they may initiate or promote immune reactions (14).

Regulation of NK cell activation is another important issue in the context of NK cell subsets. For example, $CD56^{bright}$ cells constitutively express the high-affinity heterotrimeric IL-2 receptor complex, which provides this subset an advantage of responding to very low concentrations of IL-2 (7). IL-2-induced activation of $CD56^{bright}$ cells results in the production of relatively large amounts of IFN-γ, which can shape the Th1 immune response (28). Once activated, $CD56^{bright}$ cells are as cytotoxic as the $CD56^{dim}$ subset (30). The activation of $CD56^{dim}$ cells is a very complex balance of activating and inhibitory signals. For example, when NK cells are engaged with MHC class 1 molecules, the inhibitory killer immunoglobulin-like receptors deliver a signal that prevents the NK cell from killing the target. In contrast, a number of activating receptors are present on NK cells. A full description of activation regulation is beyond the scope of this paper so the readers are directed towards excellent recent reviews on the topic (6, 7). Needless to say, the decision for an NK cell to lyse its target must ultimately mean that activating signals have dominated over inhibitory signals.

NK Cell Subsets – Distinct Cell Populations?

In spite of advances in our understanding of the biology of NK cell subsets, comprehension of their lineage remains an area of active research. It seems clear that NK cells are derived from $CD34^{+}$ hematopoietic progenitor cells and the known site(s) of development and process of maturation suggest that $CD56^{dim}$ cells are derived directly from the $CD56^{bright}$ subset (7). A recent study elegantly described a sequential lineage whereby $CD56^{bright}$, in contact with fibroblasts, can terminally differentiate into $CD56^{dim}$ cells (8). This study therefore suggested that the $CD56^{bright}$ subset represents an immature form of NK cell that eventually reaches the mature $CD56^{dim}$ phenotype with the right environment. Regardless of origin, clear functional differences exist between these subsets.

There is growing evidence that NK cell subsets differ in both gene and protein expression. In a comprehensive study by Hanna and colleagues (19) gene expression profiling of NK subsets revealed several novel functions. In the $CD56^{bright}$ subset, 888 genes were found to be transcribed at significantly lower levels (at least two fold) when compared with $CD56^{dim}$ cells, while 380 genes were up-regulated. Various mRNA species for membrane proteins/receptors, signal transduction, secreted proteins, transcriptional and translational regulation, apoptosis, cell cycle, and metabolism and structure were all found to be differentially expressed between the subsets. In some instances, 15-fold higher levels of expression for some species (e.g., Lymphopain, HLA-DRA, and Granzyme K) were observed in the $CD56^{bright}$ subset. Consistent with these findings, gene expression of cytolytic molecules was found to be generally higher in $CD56^{dim}$ than in $CD56^{bright}$ subsets (with the exception of Granzyme K) whereas expression of molecules involved in adhesion, migration, and cell to cell cross talk was generally higher in the $CD56^{bright}$ subset (46). In total, Wendt et al.'s analysis distinguished the two NK cell subsets in the expression of 473 transcripts (46). Some evidence also suggests that intrinsic (i.e., unstimulated) protein expression may be greater in $CD56^{bright}$ than in $CD56^{dim}$ cells (46). For example, IL-8 expression was greater in the former subset, although more work is required to understand differences in intrinsic protein production between the subsets. That NK cell subsets differ in both gene and protein expression in the unstimulated state is particularly relevant from a physical activity perspective considering that both subsets are mobilized into the peripheral circulation with acute exercise.

NK Cells and Acute Exercise

NK cells defined by the traditional phenotype (i.e., $CD3^{-}CD16^{+}CD56^{+}$ cells) seem to be the most sensitive and therefore responsive cell type to an acute bout of exercise, whether that exercise is predominantly aerobic or anaerobic in nature (36). NK cells are rapidly mobilized into the peripheral circulation most likely via multiple mechanisms including: shear stress due to a substantial increase in peripheral blood flow and a catecholamine-induced down-regulation of adhesion molecule expression (29). Although NK cells present in the peripheral blood represent a very small proportion of the body's total NK cell pool at rest (47), the striking exercise-induced increase in the peripheral pool has been linked to an enhanced immune surveillance (32). It is interesting to note, however, that during very prolonged exercise (i.e., > ~3 h), circulating NK cell counts may return to pre-exercise levels and can even drop below pre-exercise levels (16). The mechanisms for this are debatable, but clearly the exit of cells outweighs their entry into the circulation, possibly to enter sites of muscle damage, for which there is some evidence (26). One might argue that a blunted peripheral infiltration of NK cells, particularly of the more cytotoxic (i.e., $CD56^{dim}$) subset, might reflect a reduced ability to defend against pathogens. Alternatively, the exit of cells from the circulation or an inhibition of their entry could mean that these cells are remaining or trafficking to sites where they are needed to affect immune or inflammatory function. Indeed, the true health significance of exercise-induced changes in human NK cells is open for debate. Unfortunately, mouse NK cells do not express the

murine homologue of CD56 and although mouse NK cells can be subdivided based on expression intensity of CD27, which have some similarities to that of human NK cell subsets, *in vivo* studies of NK cell subset function are lacking.

NK Cell Subsets and Acute Exercise

Compared with the abundance of exercise literature that has addressed the traditional $CD3^{-}CD16^{+}CD56^{+}$ NK cell phenotype, there are only a handful of studies that have addressed how NK cells expressing different intensities of the CD56 antigen respond to exercise. Although Horn et al. (20) reported that an acute bout of incremental high intensity exercise mobilized NK cells with greater intensity of CD16/CD56 expression, compared with NK cells at rest, this study simultaneously measured the expression of CD16 and CD56 antigens and, therefore, could not truly distinguish between $CD56^{bright}$ and $CD56^{dim}$ cells. Gannon et al. (16) determined NK cell subset counts before and after a 250-km cycling road race. Blood samples were drawn 24 h prior to the race and 10 to 25 min following the race, which lasted approximately 7 h. These authors found that cell counts of both NK subsets determined following the race were actually lower than their respective pre-exercise levels (Table 1), supporting the idea of an exit of cells from the circulation with prolonged duration of exercise. In what appears to be the first report of the effect of acute exercise on NK cell subsets, Berk et al. (1) found that numbers of both $CD56^{+}CD16^{+}$ (likely $CD56^{dim}$ cells) and $CD56^{+}CD16^{-}$ (likely $CD56^{bright}$ cells) lymphocytes increased in the peripheral circulation after 1 h of treadmill running, but were below pre-exercise values after a full 3 h of running (Table 1). The latter study should be interpreted with caution, however, since the distinct NK cell subsets based on the expression intensity of CD56 was not specifically examined in this study. More recently, Suzui and colleagues (38) reported on the effects of brief, incremental exercise on NK cell subsets and found that only the proportion of $CD56^{dim}$ cells increased in response to exercise. A finding that was later confirmed by the same research team (39). The handful of studies described above demonstrates that both NK cell subsets are responsive to acute bouts of exercise. However, the inconsistencies in study design, blood sampling time, and flow cytometry methods make it difficult to interpret the findings. Moreover, only one of these studies measured subsets into the post-exercise recovery period.

In recent studies, our laboratory has addressed the issue of a differential mobilization of NK cell subsets in response to acute exercise. Unlike the majority of exercise immunology studies, our research is focused on the child and adolescent. In all of our studies, we used an exercise model consisting of 60 min duration at ~70% of maximal oxygen uptake (VO_{2max}). While this type of exercise is not consistent with most young people's habitual physical activity patterns, our objective was to induce significant physiological stress, thus maximizing the mobilization and representation of NK cells in the peripheral circulation. With this standardized approach, we confirmed a differential mobilization of $CD56^{bright}$ and $CD56^{dim}$ subsets in response to exercise, including an elevated ratio of $CD56^{bright}$ to $CD56^{dim}$ cells during the recovery period (43, 44). Whether studying male (44) or female (40) children or adolescent boys and girls (43), the $CD56^{dim}$

subset responded with greater magnitude than did the $CD56^{bright}$ subset after the 60 min of exercise, and this response was usually apparent after only 30 min of exercise. After 30 and 60 min of seated resting recovery following the exercise task, both $CD56^{bright}$ and $CD56^{dim}$ subsets had returned close to pre-exercise levels. However, while $CD56^{bright}$ cells remained slightly above resting levels, $CD56^{dim}$ cells dipped slightly below resting levels.

Based on the literature reviewed to this point, a common theme of a differential mobilization of NK cell subsets in response to acute exercise emerges. In almost every study, the $CD56^{dim}$ subset is more responsive to exercise than is the $CD56^{bright}$ subset. To illustrate this conclusion, we have calculated the effect size (ES) for each cell type's response to exercise from four of the studies discussed above (1, 40, 43, 44). Even though these studies implemented different testing protocols and flow cytometry methods, the comparison of NK cell subset responses remains valid because the comparison is made "within subjects". Table 1 provides the cell counts (mean ± SD) and the corresponding ES, which is calculated as: ES = (post-exercise cell count − pre-exercise cell count)/average SD of pre- and post-exercise cell counts. In these studies represented in Table 1, "post-exercise cell counts" were always taken after 60 min of exercise, thus the duration of activity is consistent across studies. In all these studies, the exercise intensity was also similar at ~70% VO_{2max}. The ESs were then compared using a dependent t-test. The results of this examination clearly indicate that the ESs of $CD56^{dim}$ cells were significantly ($p = 0.02$) greater than those of $CD56^{bright}$ cells, thus supporting a differential mobilization of NK cell subsets in response to acute exercise.

An alternative approach to illustrate the differential mobilization of NK cell subsets is to calculate the ratio of $CD56^{bright}$ to $CD56^{dim}$ cells. The clinical significance of the ratio or the balance between $CD56^{bright}$ and $CD56^{dim}$ cells is dis-

Table 1. Studies reporting the mobilization of NK cell subsets in response to acute exercise

	$CD56^{bright}$ cells					**$CD56^{dim}$ cells**				
Study	**Pre-exercise**		**Post-exercise**		**ES**	**Pre-exercise**		**Post-exercise**		**ES***
	MEAN	**SD**	**MEAN**	**SD**		**MEAN**	**SD**	**MEAN**	**SD**	
Timmons et al. 2007	14.7	7.1	28.1	13.3	**1.0**	153	58	353	178	**1.7**
Timmons et al. 2006	14.3	4.5	28.1	14.0	**1.5**	119	29	356	210	**2.0**
	14.8	7.8	18.5	11.3	**0.4**	184	31	347	251	**1.2**
	19.7	7.6	36.3	13.6	**1.6**	135	36	381	170	**2.4**
Timmons et al. 2006	19.1	9.3	45.0	18.5	**1.9**	173	81	537	236	**2.3**
	17.5	6.7	34.0	13.4	**1.6**	132	90	336	206	**1.4**
Berk et al. 1990	14.0	9.5	22.0	15.8	**0.6**	300	126	470	284	**0.8**
			MEAN ES		**1.2**			**MEAN ES**		**1.7**
Berk et al. 1990	14.0	9.5	30.0[1]	25.3	**0.9**	300	126	330[1]	190	**0.2**
Gannon et al. 1997	15.0	10.0	10.0[2]	7.0	**-0.6**	340	200	300[2]	200	**-0.2**

Unless otherwise indicated, values are derived from blood samples collected at rest and after 60 min of exercise expressed as cells × 10^6/L. ES, effect size calculated as (post-exercise mean − pre-exercise mean)/mean of pre- and post-exercise SD. *Indicates that ES for $CD56^{dim}$ cells are significantly larger than for $CD56^{bright}$ cells ($t=3.138$, $df=6$; $p = 0.02$). The two studies included at the bottom of the table are for comparison purposes, as values were taken from blood samples collected after either 3 h[1] or 7 h[2] of exercise.

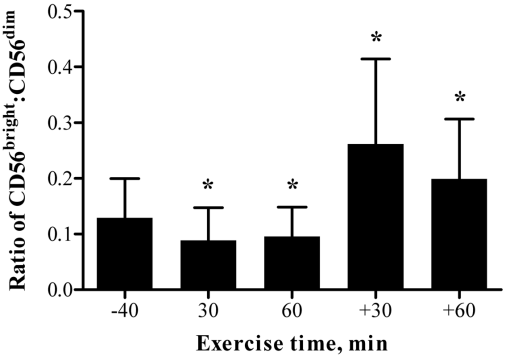

Figure 2. Ratio of $CD56^{bright}$ to $CD56^{dim}$ cells before, during, and following acute exercise in healthy children and adolescents. Values are mean ± SD. * significantly different from −40 min (rest). Subjects cycled for 60 min at ~70% of maximal oxygen uptake.

cussed later in this paper. However, a recent exercise-related study (37) found that, during sport training in healthy women, the lowest measured whole blood NK cell function (i.e., cytotoxicity) occurred concurrently with the highest blood $CD56^{bright}$:$CD56^{dim}$ ratio. In our studies of children and adolescents, we observed a slight decrease in this ratio during exercise, but a pronounced increase during the recovery period (Figure 2). Thus, the balance of NK cell subsets during recovery from physiological stress is in favour of the $CD56^{bright}$ subset. This is an important observation because the recovery period from exercise is a time when homeostatic recovery and tissue adaptation occur (25), suggesting that the $CD56^{bright}$ subset may play a role in this process (see below).

Factors that Influence Mobilization of NK Cell Subsets in Response to Acute Exercise

Notwithstanding the fact that only a few studies have addressed the impact of acute exercise on NK cell subsets, a number of factors seem to influence their mobilization. Consistent with the differential response of these subsets, the same factor may have a different effect on different subsets. In the following sections, a brief overview of some of these factors is provided.

Exercise duration and intensity

As with any marker of the immune system, the timing of a blood sample during exercise and the intensity at which the exercise is performed are important when interpreting the NK cell subset response. Our studies in children and adolescents were restricted to a total of 60 min of constant-load cycling, but there was no difference in cell counts determined after 30 or 60 min of the exercise (40, 43, 44). These findings suggest that the mobilization of NK cell subsets is relatively rapid and complete by 30 min of exercise. These findings are supported by Berk et al. (1), who found a relatively small increment in levels of $CD56^{bright}$ cells after 3 compared with 1 h of treadmill running, whereas levels of $CD56^{dim}$ cells had started to return to resting levels by 3 h of running (Table 1). In contrast, a field study found that ~7 h of road cycling resulted in NK cell subset counts that were actually lower than pre-exercise values (Table 1). These latter results must be interpreted with caution, however, since ~17 min passed from the end of the race until blood collection; it is conceivable that dramatic changes in cell counts

occurred within this time frame. Moreover, the real-life setting for this study (i.e., road racing) would mean that exercise intensity would not be kept constant, as is possible in controlled laboratory studies. Nevertheless, the limited evidence suggests that both NK cell subsets are rapidly mobilized into the circulation in response to exercise and remain at constant levels over time when the exercise intensity is held constant. This balance apparently reflects an equalization of entry and exit of these cells into and out of the peripheral circulation. Although the evidence is not strong, prolonged exercise (i.e., >3 h) may result in a net exit of NK cell subsets out of the circulation (16), as previously suggested a decade ago (17). If true, it will be interesting to identify the fate of these cells (e.g., apoptosis or tissue infiltration) and the factors that regulate these processes.

Few studies have specifically addressed the extent to which exercise intensity alters NK cell subsets. Suzui and colleagues (38) reported on the effects of brief, incremental exercise on NK cell subsets. Nine males exercised on a cycle ergometer for 5 min at each of 4 increasing intensities (50, 90, 120, and 140% of their individual ventilatory threshold), with blood samples drawn after every workload. The authors found that only the proportion of $CD56^{dim}$ cells increased in response to exercise; the proportion of $CD56^{bright}$ cells in the peripheral circulation did not change. However, because of an overall leukocytosis both $CD56^{bright}$ and $CD56^{dim}$ cell counts increased with increasing exercise intensity. In a follow-up study (39), the same authors confirmed their earlier findings by showing that in 6 males cycling for 30 min at 120% of their individual ventilatory threshold (~70% VO_{2max}) the proportion of $CD56^{dim}$ cells but not the proportion of $CD56^{bright}$ cells increased significantly. Based on these two studies, one can conclude that the redistribution of $CD56^{bright}$ cells appears to be resistant to changes in exercise intensity. However, much more work is needed to clarify how exercise intensity influences mobilization of NK cell subsets.

Training status (fitness)

To our knowledge, only one published study has determined the extent to which training status – or more specifically aerobic fitness – is associated with NK cell subsets. This study by Rhind et al. (34) reported that seven endurance-trained men exhibited a higher proportion of the $CD56^{bright}$ NK cell subset, as compared with 6 untrained men, although this difference was found in blood samples collected at rest. Whether this finding was a result of the chronically trained state or of recent training history is unclear, since Suzui and colleagues (37) demonstrated that changes in an athlete's training load acutely increases the proportion of $CD56^{bright}$ NK cells in the circulation. To this end, we are not aware of any studies that have determined whether training status *per se* influences the mobilization of NK cell subsets in response to *acute* exercise. To further explore the potential relationship between aerobic fitness and NK cell subsets, we returned to our previously published data (40, 43, 44) and performed correlation analyses on 54 boys and girls. The results are presented in Table 2. We did not find a relationship between aerobic fitness and NK cell subsets at rest, nor could we conclude that aerobic fitness was associated with the exercise-induced change in NK cell subsets (i.e., the magnitude of the exercise effect). The influence of training status on NK cell subsets therefore deserves additional investigation to clarify the influence of regular physical activity.

Carbohydrate intake

It has been known for some time that NK cells (i.e., $CD3^{-}CD16^{+}CD56^{+}$) are sensitive to carbohydrate (CHO) intake (usually in the form of a sport drink) during exercise (31). To identify the effects of CHO it was suggested that exercise must be prolonged and intense because the proposed CHO effects on NK cell redistribution in adults was due to a blunted stress hormone response mediated by main-

Table 2. Pearson correlation coefficients between aerobic fitness and NK cell subsets at rest and in response to exercise.

	VO_{2max} ($ml \cdot kg^{-1} \cdot \mathbf{BM}^{-1}$)	VO_{2max} ($ml \cdot kg^{-1} \cdot \mathbf{LBM}^{-1}$)
Resting $CD56^{bright}$ cell count	–0.11 (0.43)	–0.16 (0.25)
Resting $CD56^{dim}$ cell count	–0.10 (0.49)	–0.04 (0.78)
Resting Ratio ($CD56^{bright}$:$CD56^{dim}$)	0.09 (0.52)	–0.04 (0.80)
Resting $CD56^{bright}$ cell proportion	–0.07 (0.60)	–0.16 (0.23)
Resting $CD56^{dim}$ cell proportion	–0.04 (0.75)	–0.04 (0.78)
Exercise-induced change in $CD56^{bright}$ cell counts	–0.01 (0.95)	–0.03 (0.83)
Exercise-induced change in $CD56^{dim}$ cell counts	–0.07 (0.61)	–0.02 (0.90)

Values in parentheses are p values. VO_{2max}, maximal oxygen uptake; BM, body mass; LBM, lean body mass. The exercise-induced change in NK cell subset is taken as the difference between the cell count at 60 min of exercise (70% VO_{2max}) and at rest (i.e., Δ).

tained or increased blood glucose concentrations (31). While this explanation may be adequate for the adult response, evidence supporting this theory in the paediatric population is lacking. However, our studies did find that CHO intake attenuated the $CD56^{dim}$, but not the $CD56^{bright}$, response in young boys (44) and girls (40). In contrast, CHO intake equally attenuated both subsets in older male and female adolescents performing the same exercise (43). In pre-pubertal and early-pubertal boys, the attenuating effect of CHO on $CD56^{dim}$ cells is already visible after only 30 min of exercise whereas in late-pubertal boys the effect becomes apparent after 60 min of exercise (44). In spite of significant CHO effects on NK cell subsets in the above studies, there was no evidence of an effect on epinephrine (adrenaline), norepinephrine (noradrenaline) or cortisol – stress hormones thought to be involved in mediating the relationship between CHO intake and NK cell redistribution (31). This dissociation between changes in cell counts and stress hormones with and without CHO intake suggested a direct effect of CHO intake on NK cell subsets, possibly due to elevated blood glucose levels and associated with normal puberty. This possibility is supported by the observation that the one hormone affected by CHO intake in our studies was growth hormone (GH). We found a significant correlation between exercise-induced changes in GH and NK cell subsets in boys but this relationship was not found in girls (unpublished observations). Due to the limited evidence to date, the overall health significance of CHO effects on NK cell subset responses to exercise is unclear, but these studies need to be reproduced in adults.

Sex

In one study, female sex was formally investigated as a potential mediator of the NK cell subset response to exercise (43). Interestingly, both the $CD56^{dim}$ and

$CD56^{bright}$ subsets increased significantly more in female adolescents than in male adolescents. However, the magnitude of this enhanced response was similar between subsets as the ratio of $CD56^{bright}$:$CD56^{dim}$ cells responded in a similar fashion between the sexes. In two independent publications, we reported changes in NK cell subsets in 12-yr-old boys (44) and girls (40). Since both groups were tested under identical conditions, we were able to compare their responses to examine whether the previously observed sex-based differences in adolescents was present in younger children. Although some aspects of the NK cell response were different between the boys and girls (see ref (40) for details), the actual increase in both the $CD56^{dim}$ and $CD56^{bright}$ subsets was practically identical.

The apparent age x sex interaction in NK cell subset responses to physiological stress observed in adolescents may be relevant from a reproductive perspective. $CD56^{bright}$ cells found in the decidual tissue of early pregnancy could be important in maternal-foetal tolerance (22). Whether acute exercise or regular physical activity influences these subsets would be of considerable interest given the interest in exercise recommendations during pregnancy (45). Moreover, studies are needed to more clearly understand the potential impact of the menstrual cycle and sex hormones on NK cell subset responses to exercise. We have previously reported that the total lymphocyte pool is more responsive to exercise during the luteal phase in women taking oral contraceptives but not in non-users (41); however specific effects on NK cell subsets remain to be determined.

Puberty

As in adults, NK cells (i.e., $CD3^{-}CD16^{+}CD56^{+}$) are the most responsive cell type to exercise in children, but the magnitude of the response to strenuous exercise is lower in pre- and early-pubertal boys, as compared with men (42). Since work in our laboratory is interested primarily in exercise responses during childhood, we are particularly focused on how growth and development influence NK cell responses to exercise. To examine this issue, we recruited boys of the same chronological age but who varied in their pubertal development (44). We showed that boys at the most advanced stages of puberty demonstrated the greatest increase in the proportion of $CD56^{dim}$ during exercise, but that the increase in $CD56^{dim}$ cell counts did not vary statistically by pubertal group due to a slightly greater increase in total lymphocyte counts in the pre- and early-pubertal boys (44). However, the exercise-induced increase in both the proportion and count of the $CD56^{bright}$ subset was greatest in the boys at more advanced stages of puberty. Based on these observations, responsiveness of NK cell subsets to exercise seems to be dependent to some extent on the developmental stage of the individual.

In summary, we hope that these preliminary data will stimulate interest in further understanding the mechanisms underlying NK cell subset mobilization with acute exercise. Notwithstanding the few studies that have appropriately addressed NK cell subset mobilization with exercise, the findings suggest that several factors may be involved; in some instances the effect varies with subset (e.g., CHO intake). An important contribution to the biology of NK cells will be to elucidate functional responses (e.g., gene and protein expression) in subsets sensitive to acute exercise.

What is the Significance of Exercise-induced Changes in NK Cell Subsets?

Studies of exercise-induced changes in NK cell subsets have been descriptive in nature, and the true health significance of transient changes in these cell populations remains unclear. When assessing the significance of exercise-induced changes, we believe there are at least two possible interpretations. The first is to consider changes in NK cells representing alterations to immune function (i.e., antiviral defence). In this context, one practical consequence of exercise-induced alterations in NK cell subsets may relate to the measure of NK cell cytolytic function. A well-described phenomenon in the exercise immunology literature is a period of relative immune function depression following high-intensity exercise, consistently associated with depression of NK cell function and termed the "open window"; a period of time when the host may be at increased susceptibility to infection (32). A recent study (37) tracked changes in $CD56^{dim}$ and $CD56^{bright}$ cells over one month of competitive sports training in healthy adult females and found that the time during training with the lowest NK cell cytotoxicity corresponded to the time when $CD56^{bright}$ cell counts were highest and $CD56^{dim}$ cells remained unchanged (i.e., when $CD56^{bright}$:$CD56^{dim}$ ratio was highest). These findings support the notion that reduced NK cell function (as measured by *in vitro* cytotoxicity assays) during recovery from high-intensity acute exercise (32) and periods of intensified training (37) may be due to disproportionate changes in NK cell subsets; a high proportion of $CD56^{bright}$ cells, which have low unstimulated cytotoxicity (9), may effectively "dampen" overall killing capacity. The findings from our studies that show an increase in the ratio of $CD56^{bright}$ to $CD56^{dim}$ cells during the recovery period are further evidence that observed deficits in NK cell cytotoxic assays may be due to a disproportionate number of $CD56^{bright}$ cells in the mix. However, our studies never measured NK cell function *per se*, and we cannot therefore make this link conclusively.

Alternatively, the exercise-induced redistribution of NK cell subsets observed following the end of exercise may reflect a process of homeostatic recovery and adaptation in response to physiological stress with very little to do with immune function *per se*. Based on the understanding that $CD56^{bright}$ cells possess an enhanced capacity for cytokine production and express elevated levels of adhesion molecules integral for tissue homing, it has been suggested that $CD56^{bright}$ cells may be of particular relevance coordinating the early events of immune activation in response to endogenous tissue injury (10). In support of this hypothesis, it has been shown that $CD56^{bright}$ cells are enriched at the sites of inflammation in humans (11). Given their established roles in pathological states, it is reasonable to predict that $CD56^{bright}$ cells are mobilized as components of the normal physiological adaptation to exercise. To this end, $CD56^{bright}$ cells express an abundance of angiogenic growth factors (24), indicating the potential for these cells to contribute to angiogenesis, a hallmark physiological adaptation to regular exercise. Additional studies that measure adhesion molecule expression and cytokine and growth factor production in NK cell subsets mobilized with exercise are therefore required to further elucidate the potential role of NK cell subsets in exercise adaptation.

NK Cell Subsets and Exercise: Clinical Implications

At this time, a reminder of the maturation of the NK cell is appropriate. A recent review of the literature (1) strongly suggested that $CD56^{bright}$ NK cells give rise to a mature $CD56^{dim}$ cell, which is the prevailing circulating NK cell phenotype. Hence, if $CD56^{dim}$ cells are derived from $CD56^{bright}$ cells, then the exercise-induced mobilization of the latter subset could reflect a mobilization of "immature" NK cells. This idea is consistent with the differential mobilization of naïve and memory T cells by exercise reported by Gannon et al. (15). That an acute bout of exercise can mobilize $CD56^{bright}$ cells to the circulation, most likely from secondary lymphoid tissue where they are abundant (7), leads one to suspect an important clinical role for exercise.

Although the biological significance of NK cell subset responsiveness to exercise requires further investigation, NK cells are an important first line of defence against tumour growth, and the unique immunoregulatory properties of the $CD56^{dim}$ and $CD56^{bright}$ subsets mark them as candidates for immunotherapy of cancer (9). Whether the redistribution of $CD56^{dim}$ and $CD56^{bright}$ cells in response to exercise could be of therapeutic benefit in children recovering from cancer (12), for example, remains to be determined. In patients recovering from bone marrow transplantation, peripheral blood is reconstituted early on by $CD56^{bright}$ NK cells (21, 33) more so than $CD56^{dim}$ cells (33), which may be related to the maturation process of these cells. Thus, it has been suggested that stimulation of the $CD56^{bright}$ subset with the NK cell compartment may be a therapeutic effect to prevent relapse of residual disease (33); exercise might be an excellent strategy to achieve this effect. NK cell subsets are also implicated in a variety of diseases, ageing, and female reproduction. The proportion of $CD56^{bright}$ cells in the peripheral blood of individuals receiving coronary artery by-pass surgery, for example, tend to be lower than in age-matched controls (18). Patients with multiple sclerosis treated for 12-months with IFN-β therapy demonstrate a reduction in the proportion of $CD56^{dim}$ NK cells and an increase in the proportion of $CD56^{bright}$ NK cells (35). (The authors suggested that $CD56^{bright}$ cells may have an immunoregulatory role within the central nervous system at sites of inflammation). Finally, normal ageing is associated with a reduction in the ratio of $CD56^{bright}$ to $CD56^{dim}$ due to an expansion of $CD56^{dim}$ cells (4).

Collectively, these observations create numerous exciting opportunities to further elucidate the clinical role of NK cell subsets. Given the sensitivity of these subsets to acute exercise and their differential mobilization, it is an intriguing idea that exercise could be used in these clinical conditions as an adjunct therapy to mobilize the $CD56^{bright}$ subset. With respect to ageing, it will be important to distinguish the impact of ageing *per se* from that of physical *inactivity* on NK cell subsets, as this distinction is crucial for other immune markers (27).

SUMMARY AND FUTURE DIRECTIONS

The objective of this review was to explore the effects of acute exercise on NK cell subsets (i.e., $CD56^{bright}$ and $CD56^{dim}$) in humans with special reference to potential clinical implications of the findings. We have argued that NK cell sub-

sets display a differential mobilization in response to an acute bout of exercise, with $CD56^{dim}$ cells more responsive than $CD56^{bright}$ cells. A number of factors, including exercise duration and intensity, CHO intake, sex, and puberty, seem to influence the mobilization of these subsets, and much more work is needed to identify additional moderating factors (e.g., training status) and to understand the mechanisms of mobilization. However, we hope that the literature reviewed herein will serve as a foundation on which to pursue future studies designed to reveal the mechanisms associated with these phenomena. We also encourage exercise physiologists and immunologists to pool their efforts in future studies to further expand our understanding of how (and why) exercise impacts the biology of human NK cell subsets. Exercise, for example, may be an effective adjunct therapy to promote expansion of NK cell subsets in the development of novel immunotherapeutic approaches. In addition to their roles in pathology (e.g., arthritis), NK cells may also serve a physiological role by facilitating the adaptive processes incurred by regular physical activity. Indeed these areas, among many others, should prove to be a fruitful area of research. It is hoped that this paper will spark new research into the biology of NK cells and exercise.

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