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REVIEW

Increased airway inflammatory cells in endurance athletes: what do they mean?

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Summary

Background Inflammatory cells are increased in the airways of endurance athletes, but their role in causing exercise-induced respiratory symptoms and bronchoconstriction, or their possible long-term consequences, are uncertain.

Aim To put the results of athlete studies in perspective, by analysing the pathogenesis of airway cell changes and their impact on respiratory function.

Results Athletes of different endurance sports at rest showed increased airway neutrophils. Elite swimmers and skiers also showed large increases in airway eosinophils and lymphocytes, possibly related to chronic, exercise-related exposure to irritants or cold and dry air, respectively. Post-exercise studies reported variable responses of airway cells to exercise, but found no evidence of inflammatory cell activation in the airways, at variance with exercise-induced neutrophil activation in peripheral blood. The increase in airway inflammatory cells in athletes can result from hyperventilation-induced increase in airway osmolarity stimulating bronchial epithelial cells to release chemotactic factors. Hyperosmolarity may also inhibit activation of inflammatory cells by causing shedding of adhesion molecules, possibly explaining why airway inflammation appears 'frustrated' in athletes. Data on exhaled nitric oxide are few and variable, not allowing conclusions about its usefulness as a marker of airway inflammation in athletes, or its role in modulating bronchial responsiveness.

Conclusions The acute and long-term effects of exercise on airway cells need further study. Airway inflammatory cells are increased but not activated in athletes, both at rest and after exercise, and airway inflammation appears to regress in athletes quitting competitions. Altogether, these findings do not clearly indicate that habitual intense exercise may be detrimental for respiratory health. Rather, airway changes may represent chronic adaptive responses to exercise hyperventilation. An improved understanding of the effects of exercise on the airways will likely have a clinical impact on sports medicine, and on the current approach to exercise-based rehabilitation in respiratory disease.

Keywords adhesion molecules, airway hyperosmolarity, airway inflammation, endurance training, hyperventilation, nitric oxide

Introduction

Exercise-related respiratory symptoms are frequent in athletes, and a low exercise tolerance is common in asthmatic patients. The issue of exercise-induced changes in airway cells was initially addressed with regard to the pathogenesis of exercise-induced bronchoconstriction (EIB). Subsequent studies in athletes and experimental models, however, underlined that changes in airway cells may occur in athletes independent of symptoms or spirometric changes, raising questions on their pathogenesis and possible consequences.

Although bronchodilatation occurs in healthy and asthmatic subjects during exercise [1, 2], bronchoconstriction can occur during early recovery after exercise. Accordingly, EIB is defined as a post-exercise decrease in forced expiratory volume of 1s (FEV_1) = 10% from the baseline value [3]. The main pathogenetic theory on EIB is that exercise hyperventilation causes drying of the airways, thus increasing the osmolarity of the airway surface lining [4, 5]. The osmotic stimulus would make bronchial epithelial cells shrink and release inflammatory mediators, causing airway smooth muscle contraction [5]. Alternatively, EIB could reflect decreased airway calibre secondary to vascular engorgement at airway rewarming after exercise [6]. Experimental hyperventilation with dry and cold air causes bronchoconstriction [5], with a major importance of dryness as opposed to cooling. Indeed, airway cooling may antagonize bronchoconstriction [6, 7], but could also affect lung surfactant and destabilize small airways [8].

The functional and cellular events triggered by exercise hyperventilation have been studied in experimental models. Hyperventilation with dry air caused hyperosmolarity of airway

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surface lining [9] and bronchoconstriction [10]. Repeated hyperventilation challenges caused epithelial damage with eosinophil and neutrophil influx and increased peptidoleukotriene concentrations in bronchoalveolar lavage fluid (BALF) [11]. In cultured human bronchial epithelial cells, exposure to a hyperosmolar medium or cooling–rewarming triggered an inflammatory cascade by increasing the expression of IL-8 and RANTES partly through the activation of p38 MAP kinase [12, 13]. Therefore, both hyperventilation and airway hyperosmolarity are capable to cause bronchoconstriction and inflammatory responses.

Nevertheless, the link between EIB and airway inflammation in humans, either healthy or asthmatic, remains elusive. Some studies in asthmatic patients found a positive relationship between EIB and airway eosinophilia [14] or mast cell activation [15, 16], while others did not [17–19]. Thus, the effects of exercise appear in line with the known dissociation between inflammatory and functional airway changes in asthma [20].

The biology of airway cells and inflammatory mediators is increasingly studied in athletes. Well-trained subjects can perform prolonged exercise at high workload, a feature particularly advantageous when assessing the effects of acute exercise, whereas baseline studies may yield information on chronic, training-dependent changes. Few studies are available (10 references obtained by a MedLine search of the terms: 'airway inflammation' and 'athletes' in July 2002), but all reported airway inflammation of variable degree and type in competitive athletes of different endurance sports studied at baseline. However, signs of airway inflammation in athletes did not correlate with exercise-related respiratory symptoms, bronchial hyperresponsiveness [21], or functional alterations, such as exercise-induced hypoxaemia developing at very high workload [22]. Therefore, increased numbers of inflammatory cells in the airways in athletes seem not necessarily to be associated with major clinical or functional alterations.

This review addresses the question of the physiological meaning of exercise-related airway changes in athletes. Exercise-induced changes in exhaled nitric oxide (NO) are also reviewed, due to its potential usefulness as a non-invasive marker of airway inflammation. NO is also a mediator of non-adrenergic, non-cholinergic (NANC) bronchodilating parasympathetic nerves, and may modulate bronchial responsiveness [23], a possibly relevant issue in the pathogenesis of EIB. Finally, a tentative perspective will be outlined, to summarize current knowledge and indicate possible questions to be addressed by future studies.

Studies on respiratory symptoms and airway cells in endurance athletes

Elite athletes of summer [24–28] and winter [24, 29–31] sports show a high prevalence of exercise-induced respiratory symptoms and/or spirometric alterations. The increased risk for EIB in athletes is believed to be linked to exercise hyperventilation, through enhanced airway exposure to allergens and pollutants in summer sports, and dry and cold air in winter sports [32]. The relevance of environmental factors led to question the opportunity to diagnose asthma in athletes with exercise-related symptoms [32]. Indeed, 78% of athletes who were EIB-positive after a sport- and environment-specific exercise test turned negative when the test was repeated in the laboratory [33]. Furthermore, exercise-related respiratory symptoms poorly predicted EIB [31, 34].

Prevalence of bronchial hyperreactivity (BHR) was higher in athletes than in sedentary controls, and highest in subjects exposed to cold (skiers) or humid (swimmers) air during exercise [24]. The latter result is surprising, given that high humidity of inspired air together with low exposure to allergens would be expected to decrease the risk of exercise-related respiratory symptoms [35]. However, swimmers are chronically exposed to irritant compounds used as water disinfectants, an effect further enhanced by inadequate ventilation of indoor pools [36, 37] and particularly relevant in elite athletes, who train several hours per day. In runners, the risk to develop asthma was also increased, and BHR and asthma resulted especially frequently among atopic subjects [25, 26], possibly in relation to increased exposure to airborne allergens and pollutants.

Studies on airway cells and inflammatory markers in endurance athletes explored the possible association between airway inflammatory cells and exercise-induced respiratory symptoms. An important feature of endurance exercise is that it causes a systemic inflammatory response [38], which includes mobilization and activation of neutrophils [39, 40], and a complex pattern of pro- and anti-inflammatory mediator/cytokine release, likely related to muscle damage and intense stress [41]. Leucocyte progenitor cell counts in peripheral blood are increased in endurance athletes compared to sedentary controls, possibly reflecting heightened leucocyte turnover [40]. The intensity of exercise-induced inflammation progressively decreased in sedentary subjects undergoing training [42]. Whether exercise affects the airway and systemic compartments similarly, and the possibility that training may cause adaptations at the airway level, are unexplored issues.

The following sections will summarize the results obtained in athletes on: (a) airway cells, (b) airway inflammatory mediators and (c) adhesion molecules expressed by airway inflammatory cells.

Airway cells

Table 1 reports the results of studies on airway inflammatory cells in athletes. Studies differed for sport examined, methodology, assessment of post-exercise changes, and exercise environment (field vs. laboratory studies). The available information is far from providing a complete picture, but does highlight some features common to different studies.

First, the degree and type of airway inflammation found in athletes under resting conditions varied according to the sport tested. Cross-country skiers showed increased total cell and lymphocyte counts in BALF [43] and lymphoid aggregates [44], and increased inflammatory cells (T-lymphocytes, macrophages, eosinophils, neutrophils) in endobronchial biopsies [21]. There was also evidence of airway remodelling, i.e. increased tenascin expression in the basement membrane, but bronchial biopsy findings did not correlate with BHR, atopy, or symptoms of asthma [21]. The peculiar features of 'ski asthma' are further underlined by the lack of effect of inhaled steroid treatment on airway cells or respiratory symptoms [45].

Analysis of induced sputum in non-asthmatic amateur runners showed increased cellularity and marked neutrophilia,

Table 1. Studies on airway cells in athletes

Author, year (ref)	Athletes tested	Methods	Post-exercise data	Main results
Sue-Chu et al. 1999 [43]	Cross-country skiers	BALF	No	↑ total cell, lymphocyte, and mast cell counts; inflammatory markers not ↑
Karjalainen et al. 2000 [21]	Cross-country skiers	Bronchial biopsy	No	Compared to controls, ↑ T-lymphocytes (43-fold), macrophages (26-fold), eosinophils (twofold), PMN (twofold) in bronchial mucosa. No relationship between airway cells and BHR, symptoms or atopy
Sue-Chu et al. 2000 [45]	Cross-country skiers after inhaled steroid treatment	BALF, bronchial biopsy	No	No change in airway inflammation after budesonide treatment (800 μg/day, over 20 weeks), variable effect on symptoms
Helenius et al. 1998 [28]	Elite swimmers	Induced sputum	No	↑ PMN and eosinophil differential counts compared to controls; ↑ inflammatory markers. Eosinophils = 4% in 21% of swimmers
Helenius et al. 2002 [47]	Elite swimmers 5-year follow-up	Induced sputum	No	Eosinophil differential counts ↑ over time in athletes continuing competitions, decreased in athletes who quit
Morici et al. 2001 [48]	Swimmers training outdoors	Induced sputum	Yes	Baseline: ↑ PMN differential counts (44 ± 22%; sedentary controls: 10 ± 6%), no ↑ in eosinophils; 5-km swimming in outdoor pool: no change; 5-km swimming in the sea (hypertonic environment): slight ↑ in eosinophils
Bonsignore et al. 2001 [46]	Marathon runners	Induced sputum	Yes	Baseline: ↑ PMN differential counts (79 ± 9%); post-marathon: further ↑ PMN (91 ± 4% of total cells)
Wetter et al. 2002 [22]	Endurance athletes with EIAH	Induced sputum	Yes	Baseline: PMN differential counts: 37% (range 13–49, no control samples); post-exercise: unchanged after placebo or anti-inflammatory drugs

BALF, bronchoalveolar lavage fluid; PMN, polymorphonuclear neutrophil; BHR, bronchial hyper-reactivity.

but no increase in eosinophils or lymphocytes [46]. Elite swimmers, instead, showed increased neutrophil and eosinophil counts in induced sputum, and frank airway eosinophilia, i.e. > 4% of total cells, in one out of five subjects [28]. Airway inflammation and asthmatic symptoms improved in swimmers quitting competitive training, but tended to worsen in athletes who continued their sport career [47].

It is difficult to estimate the respective role of endurance exercise versus airway exposure to environmental factors in increasing airway inflammatory cells in athletes. We studied a group of swimmers habitually training in an outdoor pool, and for this reason chronically exposed to low concentrations of chlorine derivatives [48]. In this group of athletes, induced sputum showed low differential (< 1%) eosinophil counts. Conversely, neutrophil differential counts were increased compared to sedentary controls, and similar to those reported in elite swimmers [28, 47]. We speculate that increased neutrophils in the airways may be a direct consequence of endurance training, as they were found in all athletes tested, irrespective of practised sport. Conversely, increased eosinophils and lymphocytes may depend on environmental exposure to factors such as chlorine compounds in swimmers, or dry and cold air in skiers.

Somewhat different results were reported by a recent study in athletes developing exercise-induced arterial hypoxaemia (EIAH) at high workload. Airway inflammatory cells were not increased at baseline, and neutrophil differential counts in induced sputum were lower compared to other studies. These data are difficult to interpret, as no control data were reported [22].

The second point is that few studies analysed the airway response to acute exercise. After dry and cold air exposure, normal subjects alternating rest and moderately intense exercise for 2 h showed increased granulocyte and macrophage counts in BALF compared to the same experiment under standard indoor conditions [49]. This study, however, did not analyse the response to exercise in detail, and there are no comparable data on the acute effects of exercise in cross-country skiers. In runners after a marathon race, airway neutrophils accounted for 90% of cells in induced sputum [46], in the absence of post-race respiratory symptoms or spirometric changes. Instead, airway cell counts and composition did not change significantly after a 5-km trial in an outdoor pool in swimmers [48]. After a 5-km race in the sea, a condition of hypertonic airway exposure during exercise, the same swimmers showed slightly increased eosinophil and lymphocyte differential counts in induced sputum [48]. Finally, occurrence of arterial hypoxaemia at high workload was not associated with changes in airway cells [22]. Again, this study was different from other athlete studies, as EIAH-positive subjects underwent an incremental exercise test of limited duration in the laboratory. Altogether, the changes in airway cells after exercise were variable. More studies are needed to ascertain whether airway inflammatory cells can be affected by type, intensity, duration, or environmental conditions of exercise.

In summary, athletes of endurance sports showed increased numbers of inflammatory cells in bronchial biopsies, BALF or induced sputum. Because increased neutrophils were found in all studies, irrespective of sample type or sport activity, they can be interpreted as possibly secondary to endurance training. Increased eosinophil and lymphocyte counts were found in swimmers and skiers, respectively, suggesting a major effect of environmental factors in increasing these cell types. Further studies are necessary to define: (a) the response to acute exercise, and possible dose–response effects in relation to intensity and duration of the test; (b) the role of environmental factors in

determining different airway cell patterns; (c) the time-course and features of training-induced adaptations; and (d) long-term effects of exercise training, and their possible impact on respiratory health.

Markers of airway and systemic inflammation

Markers of inflammation in the airways can hint about the activation state of inflammatory cells in athletes. Again, data are few but agree that increased inflammatory cells in the airways are not associated with evidence of activation, as assessed by markers of inflammation in BALF or induced sputum. This was true for experiments in normal subjects exposed to dry and cold air [49], cross-country skiers studied at rest [37] and runners studied at rest and after a marathon race [46]. Elite swimmers were the only athletes where increased concentration of inflammatory markers (eosinophil peroxidase, neutrophil lipocalin) was found in induced sputum at rest [28]. Conversely, our sample of swimmers showed no evidence of inflammatory cell activation at rest or after exercise in outdoor pool or sea [48]. More specifically, neutrophil elastase concentration in induced sputum was low at all times [48]. Therefore, the concept of 'limited inflammation', originally developed to describe the low-grade systemic proinflammatory effects of endurance exercise [38], seems applicable to the airways as well.

It is noteworthy that both inflammatory cells (neutrophils) and markers (neutrophil elastase and TNFα) increase in plasma after endurance exercise [40]. In the systemic compartment, cytokine inhibitors (IL-1receptor antagonist, soluble TNF receptors 1 and 2) and anti-inflammatory cytokines, such as IL-10, are released after exercise and account for the tight control of the inflammatory response [41]. In the airways, no inhibitory pathway has been identified yet. However, analysis of expression of adhesion molecules by airway inflammatory cells may suggest a possible explanation for the 'frustrated' airway inflammation associated with endurance exercise.

Adhesion molecule expression by airway inflammatory cells

Because morphological analysis of airway cells cannot detect subtle functional changes, we started studying the expression of adhesion molecules by airway cells. In both runners [46] and swimmers [48], expression of L-selectin by airway neutrophils decreased after exercise, while expression of CD11b/CD18 decreased in runners but was unaffected in swimmers (Fig. 1). These results further underline the difference between the systemic and airway responses, as expression of CD11b/CD18 by peripheral blood neutrophils increased after a marathon race, indicating neutrophil activation [39].

Exercise is known to mobilize neutrophils in peripheral blood, an event associated with shedding of L-selectin [50]. Accordingly, low expression of L-selectin by airway neutrophils may reflect exercise-induced changes in neutrophil kinetics. However, this explanation is unsatisfactory, a stimulus being necessary for chemoattraction of neutrophils from the bloodstream into airways. Bronchial epithelial cells were shown to release IL-8 and RANTES upon exposure to a hyperosmolar medium or cooling–rewarming [12, 13], suggesting a possible mechanism for exercise-induced leucocyte migration into the airways (Fig. 2).

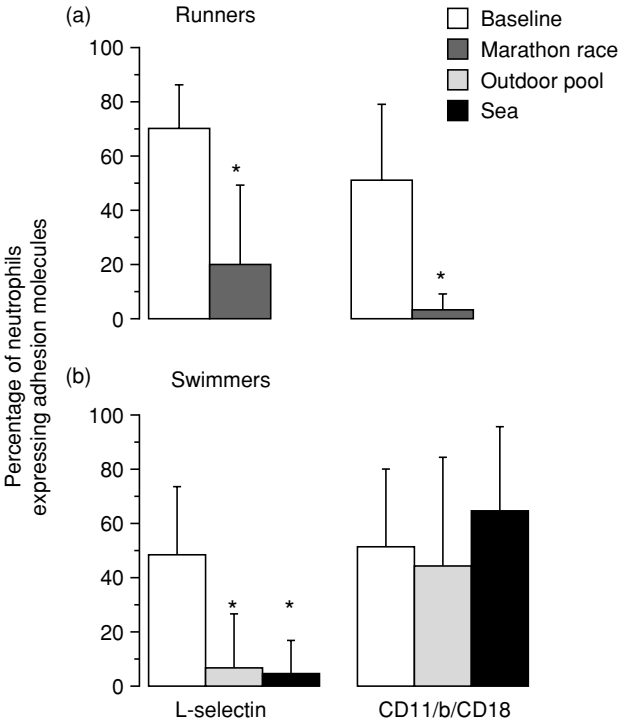

Fig. 1. Summary data on expression of adhesion molecules by airway neutrophils in runners (a, 46) and swimmers (b, 48) at rest and after exercise.

It is unclear, however, why airway neutrophils do not become activated after exercise, a 'leitmotif' of studies in athletes. Shedding of L-selectin is a possible control mechanism, as circulating neutrophils expressing low L-selectin levels tend to undergo apoptosis [51]. An alternative hypothesis comes from analysis of the possible mechanisms of the beneficial effects of hypertonic resuscitation after haemorrhagic shock [52]. Hypertonic exposure of neutrophils *in vitro* caused cell shrinkage and shedding of L-selectin, partly through p38 kinase activation [53], a picture very similar to that described for bronchial epithelial cells exposed to a hypertonic medium [12]. Neutrophils exposed to hypertonic environment became resistant to activation by endotoxin, with adhesion-independent shedding of L-selectin and inhibition of CD11b up-regulation [52]. It is tempting to hypothesize that neutrophil function might be modulated by airway hyperosmolarity induced by exercise hyperventilation. The hypothesis is attractive, as airway hyperosmolarity, known to activate bronchial epithelial cells, would trigger a self-limiting process (Fig. 2), characterized by chemoattraction of inflammatory cells whose activation state cannot proceed due to the inhibiting effects of hyperosmolar exposure.

Exhaled NO after exercise

Exhaled NO does not appear useful as a marker of airway inflammation in athletes, as normal exhaled NO values have been found in skiers with 'ski asthma' [54], competitive swimmers [48] or amateur runners [46] tested under baseline conditions.

Post-exercise changes in exhaled NO were variable, but support the possibility that exhaled NO might be related to changes

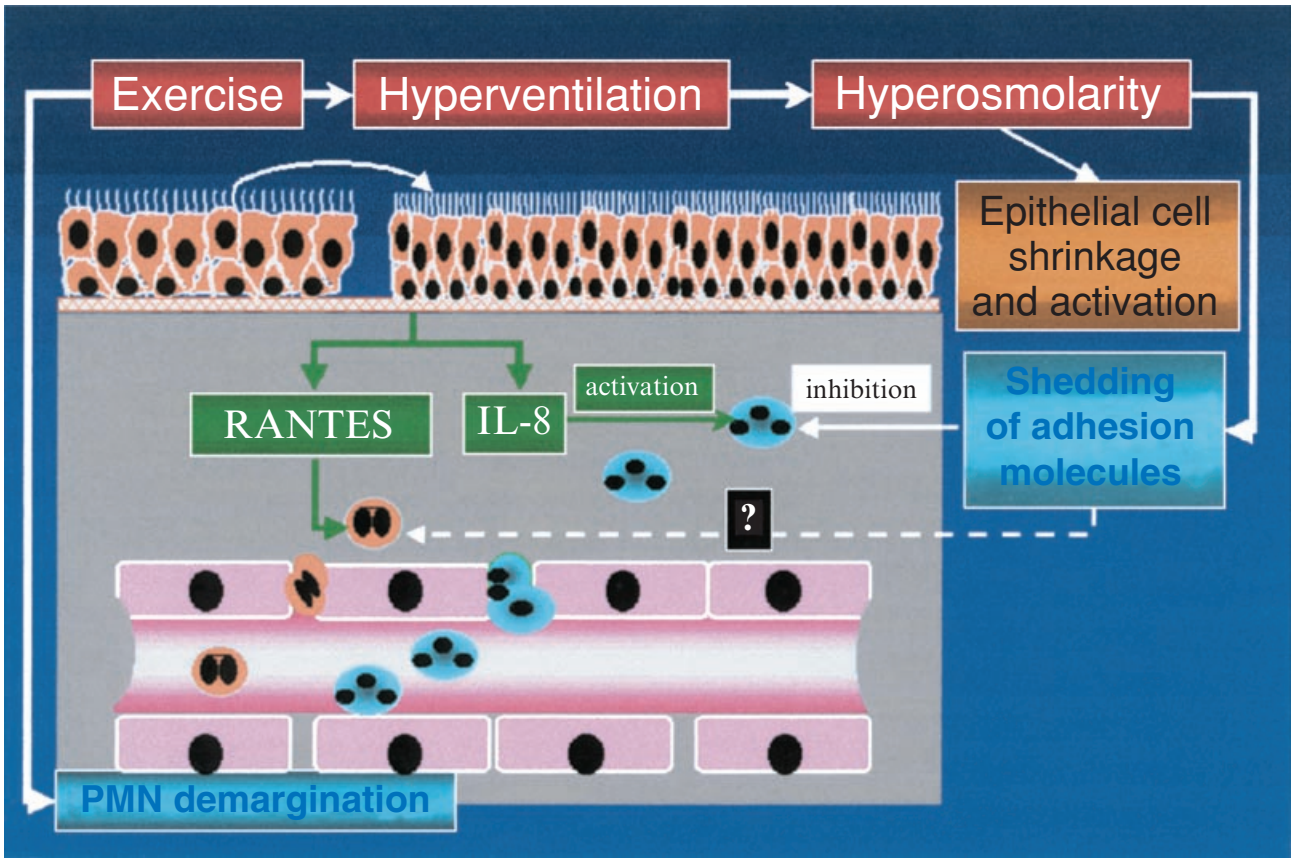

Fig. 2. Schematic sequence of cellular events possibly triggered by exercise hyperventilation. Hyperosmolarity of the airway surface layer causes osmotic changes (shrinkage) and activation of bronchial epithelial cells, with subsequent release of IL-8 and RANTES and chemoattraction of neutrophils and eosinophils, respectively [12, 13]. Demargination of neutrophils (PMN) occurs in the peripheral circulation early during exercise, and is associated with shedding of L-selectin [50]. Shedding of adhesion molecules (L-selectin, CD11b) can be induced by hypertonic exposure of PMN *in vitro* [54, 55], suggesting that a similar response may occur in airway PMN exposed to hyperosmolar environment, possibly explaining why these cells do not undergo activation (see text). It is not known whether eosinophils may also be susceptible to the inhibiting effects of airway hyperosmolarity (broken arrow). This hypothetical sequence of events may explain the apparent discrepancy between increased inflammatory cells without concomitant increase in inflammatory mediators in the airways of athletes after exercise.

in airway inflammatory cells. In non-asthmatic runners after a marathon race, exhaled NO concentration was twice the value recorded at rest, and associated with intense airway neutrophilia [46]. In swimmers after a 5-km trial or race, exhaled NO concentration was about half the baseline value when exercise was performed in the outdoor pool, and same as baseline after the sea race [48]. Airway cells were unchanged after the outdoor pool trial, while the difference in exhaled NO between outdoor pool and sea experiments could be accounted for by the mild airway eosinophilia documented after the sea race. In athletes with EIAH, airway cells were unaffected and exhaled NO did not change over 40-min recovery after exercise to exhaustion [22]. Unfortunately, NO can be released by many cell types, complicating the interpretation of results. In addition, environmental conditions and timing of postexercise NO measurements differed among studies.

Exhaled NO may also reflect modulation of bronchial responsiveness. Some data suggest that, for the same workload, well-trained athletes release more NO than sedentary subjects during exercise [55]. Another report found increased NO production in athletes associated with a higher workload compared to that attained by untrained subjects, suggesting that NO was predominantly affected by exercise intensity rather than by training status [56].

In normal subjects, but not in patients with asthma, NO concentration increased over baseline during 30-min recovery after exercise [57], suggesting a role of endogenous NO in post-exercise modulation of bronchial tone. Similar data were reported for the exhaled NO response to hyperosmolar challenge with mannitol in normal subjects and asthmatics [58]. A bronchodilating effect of NO may be especially important in winter sport athletes, as a decreased production of NO at low temperature [59] may predispose to EIB [59, 60].

In athletes, data are still insufficient to assess whether post-exercise exhaled NO may correlate with a bronchodilating action. Spirometry was normal at the time exhaled NO was increased after a marathon race, supporting a possible protection against bronchoconstriction exerted by endogenous NO in the presence of intense airway neutrophilia [46]. Similarly, the higher exhaled NO concentration after swimming in the sea compared to the outdoor pool [48] could indicate a protective effect of NO against bronchoconstriction secondary to hypertonic exposure during exercise [11]. In asthmatic patients, the analysis of exhaled NO in relation to development of

EIB gave variable results [61–63]. More studies are necessary to assess wether increased NO is associated with bronchodilatation after exercise, and on possible effects of endurance training.

In summary, further studies in athletes are needed to clarify the role of NO as a marker of airway inflammation and its intervention as a mediator of NANC-dependent bronchodilatation. Both possibilities are currently investigated, and selective NO synthase blockers will likely help to obtain more conclusive data. Future studies should be designed to overcome the technical constraint of obtaining airway cell or bronchial reactivity data, but not both at the same time, after exercise.

Conclusions and future perspectives

In summary, studies in athletes found: (a) an increase in airway inflammatory cells, with some variability among sports likely explained by different environmental exposures during exercise; (b) a 'frustrated' inflammatory process in the airways possibly related to modulation of adhesion molecules; and (c) changes in exhaled NO after exercise, reflecting changes in airway cells, modulation of bronchial responsiveness, or both.

The variable type and degree of airway inflammation observed in athletes clearly indicates the need to extend observations to other sports. Studies should include a complete functional and airway cell evaluation at baseline and after exercise, preferably after a trial specific for the sport being tested. The role played by factors such as exercise duration and intensity in modulating the type and degree of airway cell responses to exercise is still unknown. Another important question to be addressed is whether habitual training may cause long-term changes, not only in elite athletes who represent the 'high-performance' end of the spectrum, but also in the large population of amateur athletes.

The lack of activation of inflammatory cells post-exercise suggests that increased airway cells in athletes may represent a training adaptation, not necessarily implying detrimental effects for respiratory health. Most athletes are asymptomatic, and increased airway cells do not correlate with exercise-induced symptoms or presence of EIB.

We focussed our interest on airway neutrophilia, as it occurred in variable degrees in all endurance athletes tested. Peripheral blood neutrophils undergo major changes, suggesting possible, yet little explored, links between the airway and systemic compartments. Our approach is not intended to diminish the potential role of other cell types underlined by other studies; on the contrary, it indicates the need to systematically extend observations and combine them to better understand the biological response to exercise.

The present state of knowledge only allows to hypothesize the sequence of events leading to increased inflammatory cells in the airways (Fig. 2). The low expression of L-selectin by airway neutrophils suggests their influx into the airways, likely driven by chemoattractant mediators released by bronchial epithelial cells. Alternatively, the possibility that hyperosmolar changes may directly affect inflammatory cells in the airways is intriguing. We do not know whether such hypothesis may be valid for other inflammatory cells besides neutrophils, but preliminary data in swimmers indicate that airway macrophages and eosinophils also showed decreased expression of L-selectin after exercise [48]. Airway osmolarity has not been directly measured in humans. The 'sea model' may be useful to further investigate the physiology of exercise in a hypertonic environment, but differences in results after outdoor pool and sea swimming were rather small despite a likely stronger hypertonic exposure of the airways during exercise in the sea [48].

The marathon race was the only example of massive airway neutrophilia after exercise [46], possibly as a result of prolonged hyperventilation, very intense systemic neutrophil mobilization, or both. We speculate that prolonged exercise duration may be required to down-regulate CD11b expression by airway neutrophils, thus explaining the low CD11b/CD18 expression found in marathon runners (exercise lasting three hours or more) as opposed to lack of change in swimmers (exercise lasting less than 1 h).

Future studies should investigate the possible dose–response relationship between level of exercise-induced hyperventilation and airway changes. In young competitive rowers, who greatly increase minute ventilation during exercise, cellularity of induced sputum obtained shortly after 'all-out' tests correlated directly with minute ventilation during the test [64]. These short duration tests did not cause significant neutrophilia in peripheral blood, pointing to an important role of local as opposed to systemic factors (M. R. Bonsignore, unpublished observations). Functional characterization of airway cells in athletes should be a goal of future studies, as well as the identification of the stimulus responsible for their increase. Daily sessions of intense exercise are likely to affect airway cells, but the effects of training are still unknown.

The issue of EIB, and its relationship with airway inflammatory cells and endogenous NO should be further addressed. Our preliminary data suggest that an increase in airway inflammatory cells is not associated with enhanced bronchial responsiveness in non-asthmatic athletes. In marathon runners at baseline, response to methacholine was assessed after a period during which deep breaths were avoided, a condition known to favour bronchoconstriction in normal subjects [65]. After a period devoid of deep inspirations, well-trained runners showed blunted responses to methacholine compared to sedentary controls [66], indicating a low susceptibility to bronchoconstriction in well trained subjects, possibly in relation to frequent episodes of exercise hyperventilation. The clinical implications of this finding could be relevant for exercise-based rehabilitation programmes in pulmonary disease, especially in patients with asthma. We speculate that the fitness status, not generally considered in standard clinical assessment, may partly account for some of the variability observed in airway cell composition and bronchial responsiveness in healthy subjects.

In summary, well-trained athletes of several endurance sports showed increased airway inflammatory cells not necessarily associated with respiratory symptoms or functional impairment. Airway inflammatory cells were not activated at rest or after exercise, suggesting a state of 'frustrated' airway inflammation in athletes. Should chronic exercise be shown to modulate the intensity of airway inflammatory or bronchomotor responses to provocative stimuli, this finding could improve treatment and rehabilitation strategies in asthma or other pulmonary disease such as COPD. Long-term consequences of elite sports are unknown, but available evidence supports the hypothesis that endurance exercise *per se* causes adaptive responses rather than airway damage.

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