Peer reviewed article

Immunomodulating mechanisms in the lower respiratory tract: nitric oxide mediated interactions between alveolar macrophages, epithelial cells, and T-cells

Ronald M Bingisser[a], *PG Holt*[b]

[a] Departement Innere Medizin, Medizinische Universitätsklinik A, Kantonsspital Basel, Switzerland
[b] TVW Telethon Institute for Child Health Research, and Centre for Child Health Research, The University of Western Australia, Perth, Western Australia

A number of immunomodulating mechanisms are necessary to prevent uncontrolled inflammation in the lower respiratory tract. Proliferative responses of immune cells are tightly controlled in both bronchi and alveoli in the healthy lung. In diseases such as bronchial asthma, there is not only a partial failure of these mechanisms, but also an immune-deviation with a propensity towards a Th2-cell involvement.

The role of alveolar macrophages (AM) controlling T- and B-cell activation in the lower respiratory tract is discussed by considering mainly published results. This review focuses on immunomodulating mechanisms exerted via cytokines, such as Interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), and Interleukin-1 receptor-antagonist (IL-1ra), prostaglandins, such as prostaglandin E2 (PGE2), and especially nitric oxide (NO).

The Th1 and Th2 concept in asthma is introduced, being the best-described mechanism of immune-deviation in the lung. The possibility of re-inducing T-cell unresponsiveness is of particular interest. The physiological immunomodulating mechanisms used by AM are explained in detail, as they offer many possibilities for therapeutic immunomodulation. Special emphasis is put on the cGMP/phosphatase dependent, reversible mechanism of NO-mediated immunomodulation and differences in the activation of NO synthases between murine and human alveolar macrophages are mentioned.

Keywords: alveolar macrophages; bronchial epithelial cells; T-cells; immunomodulation; nitric oxide; cGMP; phosphatase; interleukin-10; transforming growth factor-β

Introduction

The epithelial-lining fluid in the lower respiratory tract contains a number of different immune cells: macrophages, T-cells, B-cells, neutrophils, eosinophils, mast cells, and the occasional dendritic cell. These populations may be readily sampled by broncho-alveolar lavage (BAL), a technique originally developed for laboratory animals [1], which was later used for clinical practice and for studies in man as a relatively simple extension of fibreoptic bronchoscopy.

Alveolar macrophages (AM) account for up to 95% of cells recovered by BAL in all species tested. Among the immunocompetent lymphocytes, approximately 65% are CD4+ T cells, which are predominantly of the activated/memory phenotype [2].

How these T cells are regulated was poorly understood for many years, because it could not be explained how they are normally prevented from reacting to irrelevant antigens in inspired air, while maintaining their capacity to respond to pathogens. Evidence from the last few years implicates the AM in the down-regulation of local lymphoid cells under normal steady-state conditions in the alveolus [3]. Epithelial cells are the primary candidates for the control of immune cells in the bronchial compartment [4, 5]. Epithelial cells may also be involved in mechanisms leading to immune-deviation as observed in the T helper 2 (Th2) response in bronchial asthma [4].

The respiratory tract: an immune-priviledged site?

As the respiratory tract is continuously exposed to organisms or particles of the environment, there is a need for appropriate defense mechanisms to prevent injurious and infectious processes. Most of the clearance of small inhaled particles and microorganisms reaching the periphery of the lung relies on the phagocytic system [6]. Macrophages are the primary phagocytes in the healthy lung. They reside in the interstitial spaces of the bronchi and alveoli, in the vascular compartment, and in the alveolus. Through the release of numerous secretory products and the expression of several surface receptors the macrophages interact with other cells and molecules [7].

Alveolar macrophages as potent modulators of the immune response

In vitro immunomodulating function of alveolar macrophages

Many in vitro studies examined the role of AMs in pulmonary immunity, and in particular their capacity to support T- and B-cell activation. Though interspecies differences were observed, these studies consistently indicate that AMs are inefficient in providing accessory cell activity in vitro compared to macrophages derived from other sources or monocytes [8]. In fact, AM have been shown to be efficient suppressors of T-cell activation and in antibody production by B cells [9]. This is especially the case at a high AM/T-cell ratio. Therefore, AM are incompetent in presentation of antigens to T-cells.

On the other hand, dendritic cells (DC) in the bronchial and alveolar walls are potent antigen-presenting cells [10]. At low AM/T-cell ratios some stimulatory effect may be present owing to the presence of DCs which can not be completely excluded in an AM preparation [10]. Electron microscopic examination of normal lung fixed by intravascular perfusion in the inflated state revealed that the majority of AM are localised in recesses at the alveolar septal junctions. In this position, AM are in intimate association with the alveolar epithelial surface, and are effectively separated by as little as 0.2 μm from underlying interstitial spaces which contain the periperal lung DC population. A similar juxtaposition of airway intraepithelial DCs was demonstrated with underlying submucosal tissue macrophages [11]. This potent downmodulation of antigen-presenting capacities of DCs in the bronchial and alveolar environment by the respective macrophage population was shown to be reversible by cytokines [12]. Thus, a potential mechanism for the *upregulation* of local T-cell responsiveness during acute inflammation is the secretion of cytokines such as GM-CSF, IL-4, TGF-β, and TNF-α by mesenchymal cells and/or macrophages.

Therefore, not only T-cells, but also DCs are maintained in a downmodulated state in the airways, tightly controlled by high numbers of nearby macrophages.

Effect of in vivo depletion of alveolar macrophages on the pulmonary immune response

- By limiting dilution analysis, pulmonary T cells isolated by bronchoalveolar lavage, exhibit a markedly reduced capacity to proliferate compared to peripheral blood T cells. However, intratracheal inoculation of liposomes containing dichloro-methylene-diphosphonate (DMDP) at a dosage shown to eliminate the majority of resident AM rapidly restores the immune competence of these lung T-cell populations [9].
- When antigen is delivered in normal animals as a bolus via the intratracheal route, virtually no response is elicited. When the same amount of antigen is identically administered to animals that were previously depleted of AM, local antibody production in pre-primed animals exposed to antigen aerosols is markedly increased [9].
- After in vivo depletion of AM by the methods described above, the antigen presenting capacity of lung dendritic cells is enhanced more than fourfold. This was shown to be a function of time post DMDP-liposome administration, with the maximal effects of AM elimination occurring within 24 h [11].

Nature of the immunomodulating mechanisms exerted by alveolar macrophages

The factors involved in T-cell and DC downmodulation by AM are partly known: In mice, nitric oxide (NO) produced by AM is the major source of this immunomodulation [3].

Several other immunomodulating factors produced by AM have been isolated, the most important being prostaglandins and cytokines.

Prostaglandin E2 (PGE2) was the first macrophage-derived immunomodulator to be described. Increased production of PGE2 by monocytes appears to be a pivotal mechanism in post-trauma immunomodulation [13]. PGE2 enhances peripheral blood lymphocyte IL-10 transcription and protein production [14] which per se offers possibilities for potent immunomodulation (see below). Finally, PGE2 has been described as deactivating AM and T-cells, and it is produced by AM [15].

An IL-1 inhibitor that competitively blocks

the IL-1 binding on its receptor was found to be the *IL-1 receptor antagonist* (IL-1ra) [16]. However, IL-1ra does not inhibit human T-cell proliferation induced by mitogens, soluble antigens or allogeneic determinants [17]. IL-4 and IL-10 have been shown to reduce the production of metalloproteinases by human alveolar macrophages [18, 19], but there is no evidence that IL-4 is produced by alveolar macrophages to suppress T-cells functions; conversely, PGE2 production by AM is inhibited by Th2 cytokines such as IL-4 [20].

TGF-β and *IL-10* have been shown to inhibit the secretion of pro-inflammatory cytokines and possibly the proliferation of T-cells [21, 22]. Another possible immunomodulating mechanism for both cytokines is a reduced rate of apoptosis in human AM, thus indirectly enhancing AM-mediated inhibition of T-cell proliferation: Alveolar macrophages from normal human volunteers undergo apoptosis after a life span of up to 100 days [23]. Upon activation with bacterial products there is a considerable increase in this basal rate of apoptosis [24]. Apoptosis is regulated by the presence of cytokines in particular, IFN-γ increases the rate of apoptosis, IL-10 and TGF-β decrease it [24]. Because AM are pivotal regulators of local immunological homeostasis, AM population density (which is regulated by lifespan and therefore the rate of apoptosis), is accordingly decisive for many immune-processes in the lungs. The reduced incidence of sarcoidosis in smokers may be one example of the potential consequences of an increased number of AM.

Molecular mechanisms of immunomodulation: involvement of nitric oxide

No or low output NO secretion by AM in humans?

Although many researchers have noted high level activation of rodent mononuclear phagocytes for nitric oxide synthetase type 2 (NOS2) expression and NO production with a variety of agents such as interferon (IFN-γ) and endotoxin, it has been difficult to demonstrate activation of NOS2 in human mononuclear phagocytes [25]. Two explanations for the hyporesponsiveness of the human iNOS (inducible nitric oxide synthetase) promoter to LPS ± IFN-γ were postulated: (1) multiple inactivating nucleotide substitutions in the human counterpart of the enhancer element that has been shown to regulate LPS/IFN-γ induced expression of the mouse NOS2 gene [26]; and (2) absence of a nuclear factor in human macrophages (eg, an LPS-inducible nuclear factor-kappa B/Rel complex), that is required for maximal expression of the gene [26]. However, treatment of mononuclear cells in vitro with IFN-α was found to cause a dose-dependent increase in NOS2 activity and NO production, and increased expression of NOS2 protein and mRNA expression [27]. In addition, IL-4 seems to induce an increased secretion of nitrite by human monocytes potentiated by IFN-γ and inhibited by the competitive NOS2 inhibitor, mono-methyl-arginine (MMA) [28]. Taken together, these and other data suggest that the expression of NOS2 is at least much more tightly controlled in human as compared to murine mononuclear phagocytes.

NOS2 expression in human bronchial epithelial cells

Evidence supporting an important role for the increased levels of NO in asthmatic airways is substantial, although the cellular and molecular mechanisms leading to increased NO are not known in detail. NOS2 expression in human bronchial epithelial cells was demonstrated by different groups. A recent publication stated that NOS2 up-regulated by cytokines could be part of an autoregulatory feedback loop (i.e., allergen challenge stimulates inflammatory cytokine production, which in turn stimulates NO production, and NO down-regulates cytokine production) [29]. Furthermore, asthmatic bronchial epithelial cells show increased NOS2 mRNA and protein due to transcriptional regulation via constitutive activation of STAT1 [5]. NOS2 mRNA expression decreases in asthmatic patients treated with inhaled corticosteroids. Post-translational mechanisms, such as an increase in the NOS2 substrate (L-arginine) in bronchial epithelial cells of asthmatic individuals may even enhance the local output of NO. Finally, nitration of proteins in airway epithelium provides evidence of functional consequences of increased NO [5].

Mechanisms of NO-induced immunomodulation

Which functional consequences of increased NO production are known in cells of the immune system?

- Nitric oxide *inhibits the GM-CSF-mediated maturation of rat lung dendritic cells* [11], and also the TNF-α-mediated maturation of human dendritic cells, via cyclic GMP-dependent mechanisms [30]. NO might therefore prolong the ability of human DCs to internalise antigens at the site of inflammation and thus modulate the initial steps leading to antigen-specific immune responses. This could indicate that antigen-presentation is not possible as long as NO is present, which is a novel form of immunomodulation in the lung.
- Nitric oxide *inhibits secretion of pro-inflammatory cytokines* such as TNF-α by alveolar

macrophages and in-vitro matured monocytes. For each cell population, the responses of asthmatics and healthy controls are comparable. The effect is not cytokine specific since similar results were obtained with GM-CSF [31]. Conversely, NO dose-dependently enhances IL-4 secretion by human PBMC [32] and Th2 cell clones [33]. A preferential inhibition of Th1-associated cytokines was not observed in another study [34].

– Nitric oxide *suppresses T-cell proliferation* in murine spleen cells, lymph-node cells in the rat [35] as well as polyclonal and monoclonal human T-cells [36].

Bronchial epithelial cells as modulators of the immune response

While it has long been recognised that the epithelium is disrupted in severe asthma, evidence was just recently gathered that the epithelium could be the origin of asthmatic inflammation. The predisposition to generate IgE to common environmental allergens ("atopy") is one of the strongest risk factors in asthma. However, while up to 50% of the population may be atopic, only 7–10% of the adult population have diagnosed asthma. Since allergen sensitisation most likely occurs via the inhaled route, the discrepancy between atopy and asthma deserves explanation. Local "tissue structure cell factors" are likely to play an important role in this regard [37].

Among the structural cells in the lower respiratory tract, the bronchial epithelium provides the physical barrier between the external environment and the internal tissue milieu – unlike bronchial smooth muscle cells and fibroblasts (that have deliberately been omitted from this review) – and would therefore be ideally placed for participating in the initiation, maintenance, and progression of asthma [38] [39]. Expression of IL-11 results in sub-basement membrane collagen deposition, myofibroblast proliferation, increased bronchial smooth muscle cell growth, and bronchial hyper-responsiveness similar to the remodelled airway in asthma but in the absence of eosinophilia [40]. These findings suggest that the bronchial epithelium may be capable of orchestrating much of the observed immunopathology of asthma.

Structural changes in the asthmatic bronchial epithelium

Evidence to support the view that the epithelium is abnormal in asthma is provided by overexpression of the epidermal growth factor receptor EGFR [41] and the epithelial isoform of the adhesion molecule CD44 in up to 80% of the airway epithelium [42]. Bronchial biopsy studies in atopic non-asthmatic subjects have revealed low levels of mast cell activation and eosinophil infiltration, suggesting that the lower airway Th2 response is "capped" in these subjects.

The epithelium as a source of inflammatory mediators

The bronchial epithelium in asthma expresses inducible enzymes for mediators (NOS2, COX2, 15-LO, and endothelin-1), cytokines (IL-1β, TNF-α, IL-6, IL-11, and chemokines) and growth factors (EGF, PDGF, GM-CSF, βFGF, TGF-α, TGF-β, and IGF-1) (reviewed in [43]). While these may be induced by cytokines released by inflammatory cells infiltrating the airways such as IFN-γ and TNF-α, an alternative explanation is that these epithelial pro-inflammatory genes are induced by growth factors generated as an integral component of an epithelial repair response. For example, epidermal growth factor (EGF) generated by epithelial cells can itself activate EGF-receptors on these cells which, through activation of the transcription factors STAT1 and STAT3 leads to the expression of inducible genes such as NOS2 and COX2 [41].

NO generated by the bronchial epithelium: immune stimulation, -suppression, or -deviation?

While certain studies propose that NO up-regulated by cytokines is part of an autoregulatory feedback loop during which NO down-regulates cytokine production [29], others claim that increased production of NO may be relevant to the pathology of asthma [44]. The bronchodilatory properties of NO are generally accepted as beneficial, whereas the pro- or anti-inflammatory properties of NO generated by bronchial epithelial cells have created conflicting hypotheses. One of the most frequently cited hypotheses was proposed by Barnes and Liew [4]: They argued that the development of specific NOS2 inhibitors could represent a novel therapeutic approach for asthma and other allergic diseases because NO derived from airway epithelial cells was thought to be a mechanism for amplifying and perpetuating asthmatic inflammation, through inhibition of Th1 cells and their production of IFN-γ. This would result in an increase in the number of Th2 cells and the cytokines IL-4 and IL-5 and therefore change the Th1/Th2 balance profoundly (see Th1 and Th2 paradigm below).

The role of NO in the pathogenesis of asthma

The Th1 and Th2 paradigm

The description of two subtypes of T-helper cells based on cytokine profiles by Mosmann and Coffman [45] was a major step forward in thinking about control of immune responses. They described murine T-cell clones that could be divided into either Th1 (producing IFN-γ and IL-2, but not IL-4 and IL-5) or Th2 (producing IL-4 and IL-5, but not IFN-γ). The potential relevance of Th2 responses to atopic disease is rapidly apparent since IL-4 and IL-5 could explain both IgE and eosinophilic inflammation: IL-4 unopposed by IFN-γ is essential in switching B cells to IgE synthesis [46] and IL-5 is involved in eosinophil development and survival [47].

The Th1 and Th2 concept in asthma

Assessment of mRNA expression in BAL cells from atopic asthmatics showed a predominant Th2 pattern [48], and numbers of cells expressing both IL-4 and IL-5 mRNA were correlated with measures of disease severity such as bronchial responsiveness or forced expiratory volume in 1s. In addition, IL-4 and IL-5 but not INF-γ protein levels were increased in BAL fluid from atopic asthmatics when compared to controls [49], and allergen-specific Th2 type clones could be isolated from the respiratory mucosa of atopic subjects [50]. In addition, by combining immunohistochemical staining to identify cell phenotype with in situ hybridisation for cytokine mRNA, IL-4 and IL-5 mRNA were predominantly localised to CD4+ cells in the airway mucosa from asthmatics, with lesser contributions from CD8+ cells, mast cells, and eosinophils [51]. Taken together, T helper-cell balance is skewed in favour of a Th2 response in asthma [52]. The factors that drive this Th2 response in initiating the allergic diathesis are yet to be identified in detail, and may act even in utero [53].

Involvement of NO

Asthmatic patients show an increased expression of inducible nitric oxide synthetase (iNOS) in airway epithelial cells [54] and an increased level of nitric oxide (NO) in exhaled air. It was also shown that treatment with inhaled corticosteroids reduces the amount of NO produced in the lungs [55]. Levels of exhaled NO in infants are even dependent on the atopy status of their parents, irrespective of the presence of asthma [56].

NO as a potent downmodulator of eosinophil apoptosis

It has been shown that Fas ligand-Fas receptor interactions are involved in the regulation of eosinophil apoptosis and that dysfunctions in this system could contribute to the accumulation of these cells in allergic and asthmatic diseases. NO was shown to be involved in Fas receptor resistance that might contribute to the eosinophilia associated with asthma. Therefore, it can be hypothesised that glucocorticoids, which are known to suppress NO concentrations in asthmatic patients, decrease eosinophil numbers by sensitisation of the Fas receptor to apoptosis [57].

Factors influencing T helper-cell balance

Not only disease, but also factors such as surgical stress [58], mercury and lead intoxication [59] as well as pregnancy [60] have an influence on a Th2 skew in the pattern of cytokine secretion, possibly a consequence of an increased rate of apoptosis of Th1 cells [61]. Th1/Th2 cytokine balance is also affected by antigen dose, affinity, and time of differentiation based on the strength of signaling and the length of the response [62].

Hormones such as corticosteroids [63], second messengers such as cGMP [34] – though with conflicting results [64] – prostaglandin E2 as well as NO [33] have been described as influencing T helper-cell balance. NO is known to inhibit the secretion of IFN-γ by Th1 cells [65, 64] but seems to lack capacity for inhibition of the production of IL-4 by Th2 cells [33]. Furthermore, Th1 response in mice lacking inducible nitric oxide synthetase (NOS2 knock-out mice) is massively enhanced [66]. The most potent regulators of the cytokine balance are notably cytokines. However, the extremely high redundance and the mutual influences in the cytokine network will make it very difficult to find the primary culprit of a T helper cell dysregulation.

The mechanism of NO-mediated immunomodulation: the final answer to "immune stimulation, -suppression or -deviation"?

T-cells activated in the presence of alveolar macrophages are unable to proliferate despite expression of IL-2R and secretion of IL-2. This process is reproduced by the NO generator S-nitroso-N-acetylpenicillamine (SNAP) and is inhibitable by the NO synthetase inhibitor monomethyl-arginine (MMA). Analysis of T-cell lysates by immunoprecipitation with specific antibodies and subsequent immunoblotting indicated marked reduction of tyrosine phosphorylation of Jak3 and STAT5 mediated by NO. Further studies indicated that NO-mediated T-cell suppression was reversible by the guanylate cyclase inhibitors methylene blue and LY-83583 and was reproduced by a cell-permeable analogue of cyclic GMP, implicating guanylate cyclase activation as a key step in the inhibition of T cell activation by NO [35].

Further studies showed the involvement of a yet unknown phosphatase causing dephosphorylation of nuclear STAT5 dimers after continuous exposure to NO for 4 to 16 hours. Interestingly, there was spontaneous re-phosphorylation of STAT5 if the continous NO exposure was stopped within 20 hours [36].

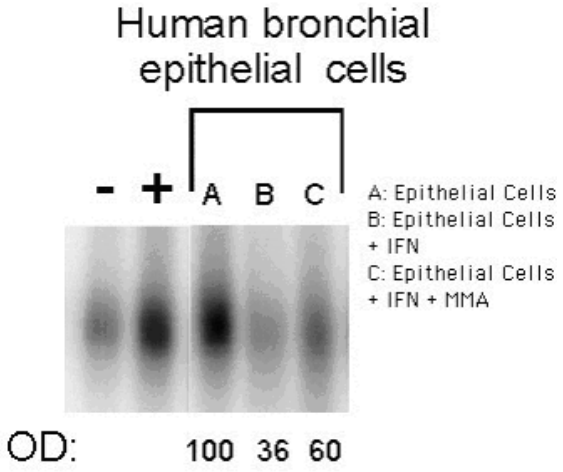
Reversible T-cell "anergy": Th2 clones unaffected?

All Th2 clones examined show a similar, NO-concentration dependent growth arrest as compared to their Th1 counterparts [36].

As Th2 cells do not appear to be resistant to growth arrest induced by NO via cGMP activated phosphatases, it seems that NO is not involved in the immune-deviation hypothesised by Barnes and Liew [4]. Conversely, NO produced by human bronchial epithelial cells inhibits T-cell proliferation irrespective of the cytokine pattern secreted by the respective clones. This is demonstrated in figure 1, where T-cell clones were stimulated with IL-2 in the presence of IFN-γ activated (and therefore NO producing) human bronchial epithelial cells [67]. As STAT5 dephosphorylation is involved in NO-induced inhibition of T-cell proliferation, and a partial reconstitution of STAT5 activity is shown in the presence of MMA, it can be concluded that growth arrest can be achieved similarly in Th1 and Th2 cells by epithelial derived NO.

Figure 1

Electro-mobility-shift assay (EMSA)of human T-cell lysates: After 24 h of culture, the T-cells were lysed and nuclear protein extracts were analysed for STAT5 activity.
–: T-cell clones deprived of IL-2 show little STAT5 activity as assessed by EMSA using radiolabelled casein-promotor as the binding site for nuclear STAT5; +:T-cells restimulated with IL-2 for 30 minutes show an enhanced STAT5 activity;
A: T-cells restimulated with IL-2 for 30 minutes in the presence of human bronchial epithelial cells show the same amount of STAT5 activity as the positive control (100% of +); B: T-cells restimulated with IL-2 for 30 minutes in the presence of human bronchial epithelial cells that were previously activated with IFN-γ show reduced STAT5 activity (36% of +) and proliferation (not shown) due to NOS2 activation and high NO production by epithelial cells; C: T-cells restimulated with IL-2 for 30 minutes in the presence of human bronchial epithelial cells that were previously activated with IFN-γ in the presence of the NO-inhibitor monomethyl-arginine (MMA) show a partial reconstitution of STAT5 activity.



Conclusion

Interactions between alveolar macrophages and T-cells in the healthy lung have been studied for the past 20 years. The immunomodulating properties of alveolar macrophages have been demonstrated in particular in the mouse and rat, but also in the human, where some of the mechanisms have not been elucidated yet. More recent evidence showed that the bronchial epithelial cell seems to possess immunomodulating properties as well. For the understanding of the pathophysiology of asthma, the immunomodulating properties of the bronchial epthelial cell are of prime interest. Among the plethora of immunomodulating mechanisms in the lung (table 1), NO is one of the best described factors. Patients with bronchial asthma show an increased level of NO in exhaled air. NO was claimed to inhibit the proliferation of Th1, but not Th2 cells, and it was therefore attractive to hypothesise that increased amounts of NO could contribute to a preferential Th2 response in allergic disease of the respiratory tract. It was concluded that specific NOS2 inhibitors could represent a novel therapeutic approach for asthma[4]. However, our own results [35–37] suggest that reversible growth arrest induced by NO is not restricted to Th1 cells. Therefore, manipu-

Table 1

Immunomodulatory cell-cell interactions in the lower respiratory tract.

Effector cell	target cell	target cell function	effector cell mechanism	stimulation (+) suppresion (–)
Macrophage	T-cell	proliferation	NO secretion	–
	T-cell	IL-10 secretion	PGE2 secretion	–
	T-cell	cytokine production	TGF-β secretion	–
	B-cell	antibody production	NO secretion	–
	dendritic cell	antigen presentation	NO secretion	–
	dendritic cell	maturation	NO secretion	–
	macrophage	apoptosis	IL-10 & TGF-β	–
Epithelial cell	T-cell	proliferation	NO secretion	–
	eosinophil	apoptosis	NO secretion	–
	macrophage	apoptosis	TGF-β secretion	–
	macrophage	TNF-α secretion	NO secretion	–
Th2 cell	macrophage	metalloproteinase	IL-4 secretion	–
	macrophage	PGE2 secretion	IL-4 secretion	–
	macrophage	apoptosis	IL-10 secretion	–
	Th2 cell	IL-12 secretion	IL-4 secretion	–
Th1 cell	macrophage	NO secretion	IFN-γ secretion*	+
	Th2 cell	IL-4 secretion	IFN-γ secretion	–
AM & epithelial	Th1 cell	IFN-γ secretion	NO secretion	–

Summary of the immunomodulatory cell-cell interactions described in this review.
*IFN-γ secretion by Th1 cells is not a directly immunomodulatory interaction between Th1 cells and macrophages. This mechanism has been included for its pivotal role activating NOS2 transcription via STAT1 in macrophages and epithelial cells.

lation of NO in the organism by means of NO inhibitors should be postponed until more detailed knowledge of their pharmacologies and toxicologies is available in order to differentiate between the effects of NO and other side effects. Hopefully, this approach could improve the predictability of the clinical outcomes of NO manipulation. As explained in this review, NO inhibitors have the potential to adversely affect the physiological immunomodulation in the lower respiratory tract. On the other hand, the use of selective phosphodiesterase inhibitors may become an attractive tool for the control of bronchial asthma, as an elevation of intracellular cGMP not only causes bronchodilation, but also contributes to a reversible growth arrest of bronchial T-cells [67].

Thus, the further elucidation of the physiological mechanisms of immunomodulation in the lower respiratory tract will lead to the discovery of better therapies for chronic lung disease.

Correspondence:
Dr. R. Bingisser
Medizinische Universitätsklinik A
Petersgraben 4
CH-4031 Basel
e-mail: rolandbingisser@hotmail.com

References

1 Myrvik P, Leake E, Fariss B. Studies on pulmonary alveolar macrophages from normal rabbits: a technique to procure them in a high state of purity. J Immunol 1961;85:128.
2 Strickland DH, Thepen T, Kees UR, Kraal G, Holt PG. Regulation of T-cell function in lung tissue by pulmonary alveolar macrophages. Immunology 1993; 80:266–72.
3 Strickland D, Kees UR, Holt PG. Regulation of T-cell activation in the lung: isolated lung T cells exhibit surface phenotypic characteristics of recent activation including down-modulated T-cell receptors, but are locked into the G0/G1 phase of the cell cycle. Immunology 1996; 87:242–9.
4 Barnes PJ, Liew FY. Nitric oxide and asthmatic inflammation. Immunol Today 1995;16:128–30.
5 Guo FH, Comhair SA, Zheng S, Dweik RA, Eissa NT, Thomassen MJ, et al. Molecular mechanisms of increased nitric oxide (NO) in asthma: evidence for transcriptional and post-translational regulation of NO synthesis. J Immunol 2000; 164:5970–80.
6 Jonsson S, Musher DM, Chapman A, Goree A, Lawrence EC. Phagocytosis and killing of common bacterial pathogens of the lung by human alveolar macrophages. J Infect Dis 1985; 152:4–13.
7 Delacourt C, Harf A, Lafuma C. Developmental aspects of alveolar macrophage functions involved in pulmonary defenses. Pediatr Pulmonol Suppl 1997;16:211–2.
8 Holt PG. Regulation of antigen-presenting cell function(s) in lung and airway tissues. Eur Respir J 1993;6:120–9.
9 Thepen T, Kraal G, Holt PG. The role of alveolar macrophages in regulation of lung inflammation. Ann N Y Acad Sci 1994; 725:200–6.
10 Holt PG, Schon-Hegrad MA, Oliver J. MHC class II antigen-bearing dendritic cells in pulmonary tissues of the rat. Regulation of antigen presentation activity by endogenous macrophage populations. J Exp Med 1988;167:262–74.
11 Holt P, Oliver J, Bilyk N, McMenamin C, McMenamin P, Kraal G, et al. Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells in vivo by resident alveolar macrophages. J Exp Med 1993;177:397–407.
12 Bilyk N,Holt PG. Inhibition of the immunosuppressive activity of resident pulmonary alveolar macrophages by granulocyte/macrophage colony-stimulating factor. J Exp Med 1993; 177:1773–7.
13 Miller-Graziano CL, Szabo G, Griffey K, Mehta B, Kodys K, Catalano D. Role of elevated monocyte transforming growth factor beta (TGF beta) production in posttrauma immunosuppression. J Clin Immunol 1991;11:95–102.
14 Huang M, Sharma S, Mao JT, Dubinett SM. Non-small cell lung cancer-derived soluble mediators and prostaglandin E2 enhance peripheral blood lymphocyte IL-10 transcription and protein production. J Immunol 1996;157:5512–20.
15 Kawano T, Ogushi F, Tani K, Endo T, Ohmoto Y, Hayashi Y, et al. Comparison of suppressive effects of a new anti-inflammatory compound, FR167653, on production of PGE2 and inflammatory cytokines, human monocytes, and alveolar macrophages in response to endotoxin. J Leukoc Biol 1999; 65:80-6.

16 Nicod LP, Galve-De-Rochemonteix B, Dayer JM. Modulation of IL-1 receptor antagonist and TNF-soluble receptors produced by alveolar macrophages and blood monocytes. Ann N Y Acad Sci 1994;725:323–30.
17 Nicod LP, el Habre F, Dayer JM. Natural and recombinant interleukin-1 receptor antagonist does not inhibit human T-cell proliferation induced by mitogens, soluble antigens or allogeneic determinants. Cytokine 1992;4:29–35.
18 Lacraz S, Nicod L, Galve-de Rochemonteix B, Baumberger C, Dayer JM, Welgus HG. Suppression of metalloproteinase biosynthesis in human alveolar macrophages by interleukin-4. J Clin Invest 1992;90:382–8.
19 Lacraz S, Nicod LP, Chicheportiche R, Welgus HG,Dayer JM. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. J Clin Invest 1995;96:2304–10.
20 Endo T, Ogushi F, Kawano T, Sone S. Comparison of the regulations by Th2-type cytokines of the arachidonic-acid metabolic pathway in human alveolar macrophages and monocytes. Am J Respir Cell Mol Biol 1998;19:300–7.
21 Cook G, Campbell JD, Carr CE, Boyd KS, Franklin IM. Transforming growth factor beta from multiple myeloma cells inhibits proliferation and IL-2 responsiveness in T lymphocytes. J Leukoc Biol 1999;66:981–8.
22 Brunetti M, Colasante A, Mascetra N, Piantelli M, Musiani P, Aiello FB. IL-10 synergizes with dexamethasone in inhibiting human T cell proliferation. J Pharmacol Exp Ther 1998;285: 915–9.
23 Bingisser R, Stey C, Weller P, Russi E, Fontana A. How do Alveolar Macrophages die? Eur Resp J 1994;7:17s.
24 Bingisser R, Stey C, Weller M, Groscurth P, Russi E, Frei K. Apoptosis in human alveolar macrophages is induced by endotoxin and is modulated by cytokines. Am J Respir Cell Mol Biol 1996;15:64–70.
25 Schneemann M, Schoedon G, Linscheid P, Walter R, Blau N, Schaffner A. Nitrite generation in interleukin-4-treated human macrophage cultures does not involve the nitric oxide synthase pathway. J Infect Dis 1997;175:130–5.
26 Zhang X, Laubach VE, Alley EW, Edwards KA, Sherman PA, Russell SW, et al. Transcriptional basis for hyporesponsiveness of the human inducible nitric oxide synthase gene to lipopolysaccharide/interferon-gamma. J Leukoc Biol 1996;59:575–85.
27 Sharara AI, Perkins DJ, Misukonis MA, Chan SU, Dominitz JA, Weinberg JB. Interferon (IFN)-alpha activation of human blood mononuclear cells in vitro and in vivo for nitric oxide synthase (NOS) type 2 mRNA and protein expression: possible relationship of induced NOS2 to the anti-hepatitis C effects of IFN-alpha in vivo. J Exp Med 1997;186:1495–502.
28 Kolb JP, Paul-Eugene N, Damais C, Yamaoka K, Drapier JC, Dugas B. Interleukin-4 stimulates cGMP production by IFN-gamma-activated human monocytes. Involvement of the nitric oxide synthase pathway. J Biol Chem 1994;269:9811–6.
29 Thomassen MJ, Raychaudhuri B, Dweik RA, Farver C, Buhrow L, Malur A, et al. Nitric oxide regulation of asthmatic airway inflammation with segmental allergen challenge. J Allergy Clin Immunol 1999;104:1174–82.
30 Paolucci C, Rovere P, De Nadai C, Manfredi AA, Clementi E. Nitric oxide inhibits the tumor necrosis factor alpha-regulated endocytosis of human dendritic cells in a cyclic GMP-dependent way. J Biol Chem 2000;275:19638–44.
31 Dinakar C, Malur A, Raychaudhuri B, Buhrow LT, Melton AL, Kavuru MS, et al. Differential regulation of human blood monocyte and alveolar macrophage inflammatory cytokine production by nitric oxide. Ann Allergy Asthma Immunol 1999; 82:217–22.
32 Kallmann BA, Malzkorn R, Kolb H. Exogenous nitric oxide modulates cytokine production in human leukocytes. Life Sci 1999;65:1787–94.
33 Chang RH, Feng MH, Liu WH,Lai MZ. Nitric oxide increased interleukin-4 expression in T lymphocytes. Immunology 1997; 90:364–9.
34 Bauer H, Jung T, Tsikas D, Stichtenoth DO, Frolich JC, Neumann C. Nitric oxide inhibits the secretion of T-helper 1- and T-helper 2- associated cytokines in activated human T cells. Immunology 1997;90:205–11.
35 Bingisser RM, Tilbrook PA, Holt PG,Kees UR. Macrophage-derived nitric oxide regulates T cell activation via reversible disruption of the Jak3/STAT5 signaling pathway. J Immunol 1998;160:5729–34.
36 Egermann U, Bingisser R. Involvement of an unknown phosphatase in the downregulation of Th1 and Th2-cell proliferation by nitric oxide (NO). Eur J Respir Dis 2000;16:5s.
37 Holt P, Macaubas C, Stumbles P, Sly P. The role of allergy in the development of asthma. Nature 1999;402:12–7.
38 Elias J, Zhu Z, Chupp G, Homer R. Airway remodelling in asthma. J Clin Invest 1999;104:1001–6.
39 Busse W, Elias J, Sheppard D, Banks-Schlegel S. NHLBI Workshop. Airway remodelling and repair. Am J Respir Crit Care Med 1999;160:1035–42.
40 Tang W, Geba G, Zheng T, Ray P, Homer R, Kuhn CI, et al. Targeted expression of IL-11 in the murine airway causes airway obstruction, bronchial remodelling and lymphocytic inflammation. J Clin Invest 1996;98:2845–53.
41 Puddicombe S, Polosa R, Richter A, Krishna M, Howarth P, Holgate S. The involvement of the epidermal growth factor receptor in epithelial repair in asthma. FASEB J 2000;14:1362–74.
42 Lackie P, Baker J, Günthert U,Holgate S. Expression of CD44 isoforms is increased in the airway epithelium of asthmatic subjects. Am J Respir Cell Mol Biol 1997;16:14–22.
43 Chung K,Barnes P. Cytokines in Asthma. Thorax 1999;54: 825–57.
44 Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howarth P, Redington A, et al. Induction of nitric oxide synthase in asthma. Lancet 1993;342:1510–3.
45 Mosmann T, Cherwinski H, Bond M, Gieldin M,Coffman R. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986;136:2348–57.
46 Del Prete G, Maggi E, Parronchi P, Chretien I, Tiri A, Macchia D, et al. IL-4 is an essential factor for the IgE synthesisinduced in vitro by human T cell clones and their supernatants. J Immunol 1988;140:4193–8.
47 Sanderson C. Interleukin-5, eosinophils and disease. Blood 1992;79:3101–9.
48 Robinson D, Hamid Q, Tsicopoulos A, Brakans J, Bentley A, Corrigan C, et al. Relationships among numbers of bronchoalveolar lavage cells expressing messenger RNA for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. N Eng J Med 1992;326:298–304.
49 Walker C, Bode E, Ln B, Hansel T, Blaser K,Virchow JJ. Allergic and non-allergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. Am Rev Respir Dis 1992;146: 109–15.
50 Del Prete G, De Carli M, D'Elios M, Maestrelli P, Ricci M, Fabbri L, et al. Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. Eur J Immunol 1993;23:1445–9.
51 Ying S, Humbert M, Barkans J, Corrigan C, Pfister R, Menz G, et al. Expression of IL-4 and IL-5 mRNA and protein product by CD4+ and CD8+ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. J Immunol 1997; 158:3539–44.
52 Kay AB. TH2-type cytokines in asthma. Ann N Y Acad Sci 1996;796:1–8.
53 Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children [see comments]. Lancet 1999;353: 196–200.
54 Howarth PH, Redington AE, Springall DR, Martin U, Bloom SR, Polak JM, et al. Epithelially derived endothelin and nitric oxide in asthma. Int Arch Allergy Immunol 1995;107:228–30.
55 Baraldi E, Dario C, Ongaro R, Scollo M, Azzolin NM, Panza N, et al. Exhaled nitric oxide concentrations during treatment of wheezing exacerbation in infants and young children. Am J Respir Crit Care Med 1999;159:1284–8.
56 Wildhaber JH, Hall GL, Stick SM. Measurements of exhaled nitric oxide with the single-breath technique and positive expiratory pressure in infants. Am J Respir Crit Care Med 1999; 159:74–8.
57 Hebestreit H, Dibbert B, Balatti I, Braun D, Schapowal A, Blaser K, et al. Disruption of Fas receptor signaling by nitric oxide in eosinophilia. J Exp Med 1998;187:415–25.
58 Decker D, Schondorf M, Bidlingmaier F, Hirner A, von Ruecker AA. Surgical stress induces a shift in the type-1/type-2 T-helper cell balance, suggesting down-regulation of cell-mediated and up-regulation of antibody-mediated immunity commensurate to the trauma. Surgery 1996;119:316–25.
59 Heo Y, Parsons PJ, Lawrence DA. Lead differentially modifies cytokine production in vitro and in vivo. Toxicol Appl Pharmacol 1996;138:149–57.
60 Saito S, Tsukaguchi N, Hasegawa T, Michimata T, Tsuda H, Narita N. Distribution of Th1, Th2, and Th0 and the Th1/Th2 cell ratios in human peripheral and endometrial T cells. Am J Reprod Immunol 1999;42:240–5.

61 Reinhard G, Noll A, Schlebusch H, Mallmann P, Ruecker AV. Shifts in the TH1/TH2 balance during human pregnancy correlate with apoptotic changes. Biochem Biophys Res Commun 1998;245:933–8.
62 Rogers PR, Croft M. Peptide dose, affinity, and time of differentiation can contribute to the Th1/Th2 cytokine balance. J Immunol 1999;163:1205–13.
63 Almawi WY, Melemedjian OK, Rieder MJ. An alternate mechanism of glucocorticoid anti-proliferative effect: promotion of a Th2 cytokine-secreting profile. Clin Transplant 1999;13:365–74.
64 Roozendaal R, Vellenga E, Postma DS, De Monchy JG, Kauffman HF. Nitric oxide selectively decreases interferon-gamma expression by activated human T lymphocytes via a cGMP-independent mechanism. Immunology 1999;98:393–9.
65 Taylor-Robinson AW, Liew FY, Severn A, Xu D, McSorley SJ, Garside P, et al. Regulation of the immune response by nitric oxide differentially produced by T helper type 1 and T helper type 2 cells. Eur J Immunol 1994;24:980–4.
66 MacLean A, Wei XQ, Huang FP, Al-Alem UA, Chan WL, Liew FY. Mice lacking inducible nitric-oxide synthase are more susceptible to herpes simplex virus infection despite enhanced Th1 cell responses. J Gen Virol 1998;79:825–30.
67 Bingisser R, Egermann U. Regulation of T-cell proliferation by the cyclic nucleotide cGMP. Swiss Medical Forum 2001; 1:21s.

Swiss
Medical Weekly

Official journal of
the Swiss Society of Infectious disease
the Swiss Society of Internal Medicine
the Swiss Respiratory Society

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising, to the current 1.537
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)

- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of our professional statistician for every article with statistical analyses

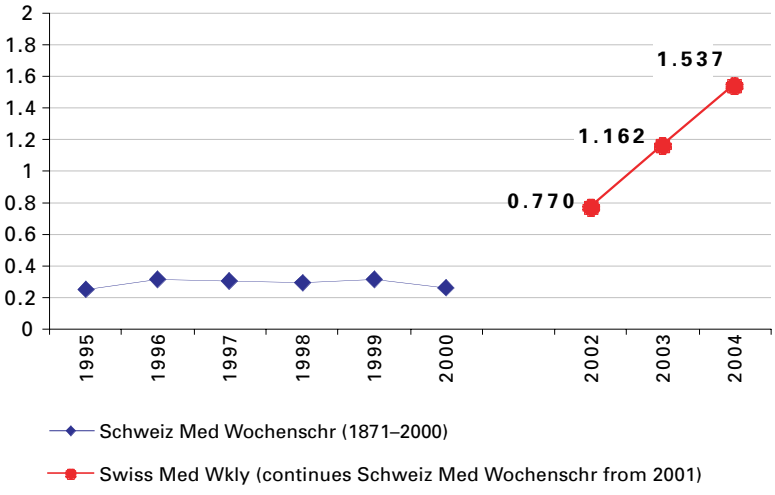
- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing
- No page charges and attractive colour offprints at no extra cost

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:
http://www.smw.ch/set_authors.html

Impact factor Swiss Medical Weekly



EMH FMH Schwabe
Editores Medicorum Helveticorum

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts: submission@smw.ch
Letters to the editor: letters@smw.ch
Editorial Board: red@smw.ch
Internet: http://www.smw.ch