Eur Respir J
1993, 6, 120–129

REVIEW

Regulation of antigen-presenting cell function(s) in lung and airway tissues

P.G. Holt

Regulation of antigen-presenting cell function(s) in lung and airway tissues. P.G. Holt.

ABSTRACT: A variety of cell populations present in respiratory tract tissues can express the function-associated molecules on their surface which are required for presentation of antigen to T-lymphocyte receptors. However, the potential role of individual cell populations in regulation of local T-lymphocyte-dependent immune reactions in the lung and airways depends on a variety of additional factors, including their precise localisation, migration characteristics, expression of T-cell "co-stimulatory" signals, responsiveness to inflammatory (in particular cytokine) stimuli, host immune status, and the nature of the antigen challenge. Recent evidence (reviewed below) suggests that the induction of primary immunity (*viz.* "sensitisation") to inhaled antigens is normally controlled by specialised populations of Dendritic Cells, which perform a surveillance role within the epithelia of the upper and lower respiratory tract; in the pre-sensitised host, a variety of other cell populations (both bone-marrow derived and mesenchymal) may participate in re-stimulation of "memory" T-lymphocytes.

Eur Respir J., 1993, 6, 120–129.

Correspondence: P.G. Holt
Division of Cell Biology
The Western Australian Research Institute for Child Health
GPO Box D184
Perth, Western Australia 6001.

Keywords: Antigen presentation
airway epithelium
class II MHC (Ia) antigen expression
T-lymphocyte activation

Received: April 30, 1992
Accepted: September 8, 1992

Aberrant immunoinflammatory responses to exogenous antigens are important aetiological factors in a large proportion of the common non-malignant respiratory diseases. The inflammatory processes which produce the characteristic pathology in these diseases, are controlled directly or indirectly by the secreted products of T-cells, and result ultimately from a chain of cellular reactions initiated by the "presentation" of inhaled foreign antigen to the T-cell receptor. This presentation event may occur locally, giving rise to immediate activation of cytokine producing memory T-cells in the airway mucosa [1], and lumen [2], as has been observed in chronic asthmatics, or systemically, particularly in the regional lymph nodes draining the respiratory tract, where initial "priming" of the naive immune system occurs following inhalation of antigens not previously encountered [3].

The immunological sequelae of these initial T-cell activation events are highly variable and can range from T-cell anergy or tolerance, to the manifestations of hypersensitivity and associated tissue pathology. The eventual outcome of individual encounters with inhaled antigens depends to a significant extent on the functional capacity of the antigen-presenting cells (APCs), which initially sequester the antigens. Recent information on the nature of APC populations in different tissue microenvironments in the respiratory tract, reviewed below, suggests that a high level of local control of T-cell activation is normally operative to protect the lung against unnecessary and/or exaggerated responses to foreign antigens.

Identification of APCs in respiratory tract tissues *in situ:* immunostaining of cells expressing class II major histocompatibility complex (MHC) immune region associated (Ia) antigen

Surface expression of Ia is a necessary prerequisite for "presentation" of antigen to the T-cell receptor, and, in general terms, the concentration of Ia on the cell surface correlates with the potency of APCs in T-cell activation [4]. Thus, potential APCs may be identified in tissue sections by immunoenzymatic staining employing monoclonal antibodies against Ia. A substantial literature is available from studies which have applied this technology to lung tissue.

Cells expressing Ia are abundant in lung tissues of all species examined, and are found both within epithelial and submucosal sites throughout the conducting airways, and within the connective tissues sur- rounding blood vessels and alveolar septal walls in the lung parenchyma. The precise identity of immuno-stained cells in lung tissue sections has been understandably controversial, in view of the difficulties associated with controlling the plane of section;

particularly with small biopsy samples from human lung. Interpretation of the human literature is further-complicated by the paucity of data on normal control tissue.

At least seven major cell types have been positively identified as expressing Ia in healthy or diseased lungs: B-lymphocytes, activated T-lymphocytes, fibroblasts, dendritic cells (DCs), and macrophages - the latter can be further subdivided (only in humans) into resident pulmonary alveolar macrophages (PAMs), infiltrating monocytes present in either extracellular fluids on the alveolar surface or within solid tissues, and mature tissue macrophages. With the exception of Ia^+T-lymphoblasts, all of these latter cells have been invoked as potential regulators of antigen specific T-cell responses to inhaled antigens, and the salient aspects of the supporting evidence for these claims is reviewed below.

Factors determining the contribution of potential APC populations to individual immune responses in the lung

The cell populations detailed above can be broadly classified as "professional" APCs (B-cells, macrophages and DCs) or "opportunistic" APCs (airway epithelial cells, Type II pneumocytes and fibroblasts). The potential for each cell population to participate in an immune induction event in the respiratory tract is determined by two sets of interacting factors: host immune status, and the physicochemical properties of the inhaled antigen.

It is axiomatic that large insoluble particulate antigens will gain only limited access to cell populations below epithelial surfaces, and hence the transmission of antigenic signals to the T-cells system from such sources requires the participation of actively phagocytic cells, which function at the level of the airway lumenal surface. Moreover, the relevant APC requires the additional capacity to migrate selectively to T-cell zones in regional lymph nodes, in order to induce a primary immune response in a naive host.

In contrast, low molecular weight soluble antigens, may be expected to be readily translocated to intra-epithelial or submucosal microenvironments *via* intracellular (pinocytic) or intercellular pathways [5], and hence gain access to all the potential APC populations listed above. This class of antigen, typified by *Der p* I allergen from the house dust mite, which leaches rapidly into epithelial lining fluids after impaction on the airway surface [6], appears particularly effective in inducing immunologically-mediated disease.

A further factor of importance, relates to the capacity of individual antigens to directly "stimulate" aspects of APC function. It is now recognized that for effective T-cell activation the APC requires to deliver two simultaneous signals to the T-cell-receptor, firstly processed antigen (peptide) complexed with surface MHC class II (Ia), and secondly a "co-stimulator" signal (or signals) supplied *via* a second group of cell surface molecules [7]. The production of co-stimulator(s) by DCs is constitutive [8, 9], whereas macrophages and B-cells produce little, unless they are pre-stimulated with natural "adjuvants", in particular bacterial cell wall antigens containing lipopolysaccharide [8]. Mesenchymal cells, such as epithelia and fibroblasts, are believed to be generally incapable of delivering an effective co-stimulator signal [10], although this issue is controversial.

Host immune status is also an important determinant in selection of appropriate APCs. It is now recognized that naive (*i.e.* non-immune) and memory-T-cells have different requirements for antigen-specific activation. In particular, with respect to soluble protein antigens, monocytes/macrophages and B-cells function very poorly in presentation of inductive signals to naive T-cells, whereas they effectively stimulate responses in memory T-cells [9, 11, 12, 13]. DCs appear unique in their capacity to present soluble protein antigens to non-immune T-cells [9] and hence prime/sensitize the naive host.

The situation with respect to the induction of primary immunity to particulate antigens is less clear, as DCs are actively endocytic, but appear non-phagocytic *in vitro* [9]. However, at the ultrastructural level, mature DCs exhibit cytoplasmic inclusions characteristic of secondary lysozomes, prompting suggestions of phagocytic activity at an earlier stage of their life cycle *in vivo* [14].

Based on the above, our current understanding of the role of the various candidate APCs is outlined below.

APC populations in normal lung

Dendritic cells

The presence of DCs in human parenchymal lung tissue was initially associated with underlying inflammatory and fibrotic disease states [15, 16]. However, they were subsequently identified as normal residents of the airway epithelium and alveolar septa in all species [17–20].

The first evidence of a role for these cells in antigen presentation to T-lymphocytes, was provided by a study on rat lung from our laboratory [21]. These experiments employed collagenase digestion to extract mononuclear cells from lung parenchymal tissue, prior to assessment of APC activity in various cell fractions of the digest. The phenotype of the principal APC in these digests was non-adherent, non-phagocytic, surface immunoglobulin (sIg^-), Ia^+ and of ultra-low density on percoll gradients, consistent with their identity as DCs [21]. These results were confirmed and extended to include an identical population in the airway epithelium [20], and similar observations have since been reported for other species including humans [18, 22–24]. A consistent finding in these studies has been the presence of an endogenous macrophage population in lung tissue digests, which inhibits DC-induced T-cell activation *in vitro* [20, 21, 25, 26]. We have suggested that the induction of T-cell immunity in the lungs is co-ordinately regulated *in vivo* by

countermanding signals from these two cell types [20, 21].

An important consideration in assessing the potential importance of DC populations in regulation of T-cell dependent immunity in the respiratory tract, is their distribution *in vivo*, and their contribution to overall expression of class II MHC (Ia) in these tissues. The general impression from the literature is that expression of Ia in the deep (peripheral) lung is extremely heterogeneous, *e.g.* [19], and even in specified pathogen free (SPF) animals the Ia^+ population in the alveolar region can include DCs, macrophages, B-cells and Type II alveolar epithelial cells. However, in a recent study with SPF rats employing dual colour immunostaining of frozen sections with a panel of monoclonal antibodies (MoAbs) specific for surface antigens on DCs and/or macrophages, the DCs were estimated to comprise up to 25% of the overall Ia^+ cell population resident in the normal lung parenchyma [27]. This figure may be an underestimate, given the likelihood that a random plane of section would in many cases reveal only part of the cell body of highly pleiomorphic DCs.

Interpretation of immunoperoxidase staining patterns in tissue samples from the conducting airways has, hitherto, been similarly complex. However, we have recently developed a novel approach to this problem, stimulated by published data on the DC population in the epidermis (Langerhan's cells) [28]. The highly developed Langerhan's cell network was only revealed by the use of epidermal sheets for immunostaining, which provide an *en face* view of intraepidermal cells. To achieve a comparable plane of section with isolated airway segments, sequential frozen sections are taken tangentially parallel to the long axis of the tube, eventually yielding fields of view through the epithelium parallel to the underlying basement membrane. Immunoperoxidase staining of Ia in such tangential sections reveals a picture which is in marked contrast to the apparently heterogeneous staining pattern seen with conventional transverse sections, demonstrating instead that virtually all Ia expression in non-inflamed airway epithelium of rats [29], and humans [30], can be accounted for by cells with the characteristic morphology of DCs.

Detailed morphometric analysis of Ia staining in the rat airways indicates an inverse relationship between airway diameter and intraepithelial DC density, which varies from 500–600 cells·mm^{-2} epithelial surface in the trachea (equivalent to published figures for the epidermis) down to approximately 50 cells·mm^2 in small airways below generation 5 [31], suggesting that DC population density relates directly to the intensity of inflammatory stimulation provided to the epithelium by the outside environment [31]. The limited information available to us on humans [30], suggests that this gradation is much less marked, possibly indicating difference in life span and hence cumulative inflammatory stimulation of the smaller airways.

Both the airway intraepithelial DCs [20] and their alveolar septal wall counterparts [27] have been shown to sequester inhaled antigen *in situ* and to be capable of processing and subsequently presenting the antigen to immune T-cells *in vitro*, thus establishing their capabilities as potential APCs *in vivo*. However, their functional capacity does not appear to be static but like cells in the mononuclear phagocyte lineage, alters radically during maturation. Based upon available data on DC populations from other tissues (reviewed in [9]), it may be assumed that the majority of lung DCs initially arrive *via* the blood as monocyte-like DC precursors, and home selectively to intraepithelial microenvironments. They mature *in situ* into Langerhan's-like DCs over a period of weeks, during which time they sample, process and "store" inhaled antigens; they eventually migrate from their epithelial environment (possibly under the influence of cytokine signals such as tumour necrosis factor α (TNFα) [32], and selectively home to T-cell zones in the regional lymph nodes [9]. Analogous to epidermal Langerhan's cells, lung DCs function relatively poorly as APCs when freshly isolated from lung tissue, but mature into extremely potent APCs during overnight culture in medium containing T-cell-derived cytokines [27, 33]. This suggests that their primary function *in vivo* is to serve as a "sentinel" cell in the respiratory tract at the epithelial interface with the outside environment, sampling incoming antigens for eventual presentation to the immune system in the regional lymph node. Their relative inability to present inhaled antigen to T-cells during the intraepithelial phase of their life cycle may be a protective mechanism, limiting T-cell-mediated damage to host epithelial tissues, which would inevitably accompany local T-cell activation [27].

The fact that these DCs constitute the only resident cell population within the normal airway epithelium which constitutively expresses Ia, strongly suggests that they constitute the "first line of defence" against inhaled antigens. As such, they are likely to play a major role in the aetiology of both allergic and infectious diseases (in particular viral), in the respiratory tract [29, 31].

Mononuclear phagocytic cells (MPC)

Substantial macrophage populations are present within both the airway lumen [34], and the lung interstitium [35, 36], and these cells are accessible for study, respectively, by bronchoalveolar lavage (BAL) and collagenase tissue digestion. A third population can be found in the airway wall and, in normal tissue, is restricted to the submucosal side of the epithelial basement membrane [27].

Studies on the APC function(s) of these MPC populations have focused principally on pulmonary alveolar macrophages (PAMs). The literature in this area has been somewhat controversial, as variations have been reported in PAM activity in *in vitro* T-cell culture systems, both between species, and within individual species (including human), employing different T-cell activation stimuli. However, employing

antigenic (as opposed to polyclonal) stimulation, particularly at macrophage:lymphocyte ratios above 1:5, PAMs from human and most animal species are actively lymphocytostatic and inhibit T-cell activation (reviewed in [37]). Interstitial macrophage populations are also inhibitory to T-cell activation *in vitro*, albeit less effectively than PAMs [20, 21, 38].

The renewal of the resident PAM population in the steady state is *via* a combination of local division of precursors (probably including an interstitial component) and recruitment of blood monocytes, the latter component increasing dramatically in response to local inflammation [39–43]). The resident PAM population, sampled at a static time point, will reflect accordingly reflect a continuum of maturation from monocyte to mature PAM. It is clear that the suppressive phenotype of the resident PAM is acquired during this maturation process, *e.g.* [44], as monocytes from most species function effectively in APC assays.

Consistent with this notion, fractionation of PAM populations, by unit gravity or density gradient sedimentation, reveals the presence of subsets which vary in activity from stimulatory to inhibitory towards T-cell activation [45–49]. The activity of these functionally distinct subsets can also be demonstrated by appropriate dose-response manoeuvres [50]. It is also relevant to note that small numbers of PAMs are able to migrate from the alveoli to regional lymph nodes [51], where they home to T-cell regions [52]. It is not known whether the signals that they provide to T-cells in that microenvironment are stimulatory or inhibitory.

However, under steady-state conditions, the net *in vitro* effect of the overall PAM population is consistently immunosuppressive [37]. Compelling evidence in support of a similar suppressive role for PAMs *in vivo* has recently been provided, from studies involving their selective elimination *in situ* by the intratracheal administration of liposome-encapsulated dichloromethylene diphosphonate (DMDP). The liposomes are avidly phagocytosed by PAM, leading to the death of the majority of these cells over the ensuing 12–24 h [53]; minimal translocation of the drug occurs into the lung or airway wall, and no cellular infiltrate is observed [53, 54]. Subsequent antigen exposure *via* intratracheal injection or aerosol inhalation reveals that PAM-depletion induces severe immune dysregulation, as the liposome-treated animals mounted large local and systemic antibody responses (including immunoglobulin E (IgE)), while untreated controls remained refractory to challenge [53–55]. In addition, if the animals had been pre-sensitized to the challenge antigen the antibody response was accompanied by massive mononuclear cell infiltration (including activated T-cells) of the connective tissues surrounding the airways and blood vessels in the lung [55].

Comparison of the performance of freshly isolated parenchymal lung DCs from intact and DMDP-liposome-treated animals in T-cell-activation assays indicated that PAM depletion is associated with large-scale upregulation of APC capacity *in situ*, demonstrable within 24 h of treatment [54]. Additionally, the *in vitro* "maturation" of APC functions, which normally occurs during overnight culture of purified lung DCs [27], can be completely inhibited by co-incubation with PAMs across a semi-permeable membrane [56]. Experiments are in progress in our laboratories to identify the cytokines which mediate these effects.

These findings suggest that down-modulation of the APC activity of DCs may represent a major pathway by which resident PAMs regulate the immunological milieu of the lung. It is of interest to note, in this context, that analysis of the distribution of PAMs on the surface of surgically removed human lungs, in which retention of these cells *in situ* had been optimally preserved by vascular perfusion-fixation, indicated that all PAMs were closely juxtaposed to alveolar septal functions [56]. Our observations, in rat lung immunostained for Ia, indicate that the majority of Ia^+ DC-like cells are located within these same alveolar septal junctional zones *e.g.* [57], *i.e.* separated from adjacent PAMs by the width of a single Type 1 alveolar epithelial cell. A similar juxtaposition occurs in the airway mucosa, where DCs and mature tissue macrophages are aligned on opposite sides of the epithelial basement membrane [27].

Epithelial cells

Ia antigen expression by ostensibly normal airway epithelial cells [58, 59] raises the question of their possible role in regulation of local T-cell responses. Several recent studies have presented evidence that human bronchial epithelial cells may upregulate T-cell activation *in vitro*. Firstly, airway epithelial cells precultured for six days in nutritionally-limiting medium have been shown to stimulate allogeneic MLR reactions [60]. However, the authors were unaware of the magnitude of potential contamination with intraepithelial DCs (and possible precursors - a further unresolved issue) which could comprise up to 10^3 cells·mm^{-2} epithelium [30, 31]; furthermore, airway epithelia are known sources of cytokines such as granulocyte/macrophage colony-stimulating factor (GM-CSF) *e.g.* [61, 62], which promote the survival of DCs *in vitro* [9]. Additionally, capacity to induce a polyclonal mixed lymphocyte reaction (MLR) reaction does not necessarily equate to capacity to induce conventional antigen-specific T-cell responses, as demonstrated by the fact that PAMs can effectively stimulate alloreactive T-cells [60, 63], while performing poorly in the presentation of protein or viral antigens [37].

A second report has demonstrated that bronchial epithelial cells can function as co-stimulants in T-cell responses induced by polyclonal mitogens or CD3-cross linking, but functioned poorly in presentation of microbial antigens, even after preculture in γ-interferon (γIFN) to upregulate Ia expression [64].

The co-stimulatory activity of the epithelial cells may reflect their capacity to produce mediators which are known to modulate the function(s) of DCs and/or macrophages [61, 62, 65].

Type II alveolar epithelial cells have recently also been recognized to constitutively express Ia [66]. Analysis of their performance in mitogen-stimulated cultures of splenocytes demonstrated their capacity to block lymphocyte proliferation, without inhibiting activation as measured by interleukin-2 (IL-2) receptor expression [67]. These effects are reminiscent of the suppression/anergy induced by exposure of specific T-cells to antigen-pulsed Ia$^+$ keratinocytes [10, 68], which is believed to result from stimulation of the T-cell receptor in the absence of the obligatory "co-stimulator" or second signal required for T-cell proliferation. No information is currently available on the activity of these cells in relation to antigen-specific T-cell activation.

Fibroblasts

Recent studies on murine lung fibroblasts, indicate the existence of two distinct subpopulations on the basis of surface expression of Thy 1 antigen. Exposure of Thy 1$^-$ (but not Thy 1$^+$) lung fibroblasts to γIFN stimulated surface Ia expression, and conferred on the cells the ability to present protein antigen to a specific T-cell clone [69]. It will be necessary to establish the capacity of these fibroblasts to present antigen to freshly isolated T-cells which have not been adapted to long term *in vitro* growth, before the significance of this observation can be further assessed.

B-lymphocytes

As noted previously, B-cells function effectively as APCs for restimulation of T-memory cells. Small numbers of B-cells are present in the lung wall and alveolar spaces in normal individuals, but there have been no reported formal studies on their APC functions after extraction from lung tissue.

Intravascular cell populations

The extensive lung vascular bed contains a substantial pool of transiently sequestered T-lymphocytes (see discussion [70, 71]), a significant proportion of which appear to be in the process of division [72]. Accordingly the theoretical possibility exists of local T-cell stimulation in response to blood-borne antigens, provided that local APCs are present. The available literature suggests four potential candidates: transiently sequestered B-cell blasts [73]; cells from the marginal monocyte pool [74]; intravascular macrophages, initially thought to be restricted to ruminants [75] but since demonstrated in humans [76]; and a subpopulation of intravascular DCs, thus far only described in rats [77]. Given the potential consequences of T-cell activation in this fragile microenvironment, the functional capacity of these cell populations appear worthy of study.

Inflammation-induced changes in lung APC populations

Dendritic cells

Recent studies in rats from our laboratory [31] have demonstrated marked increases in airway intraepithelial DC density in acute inflammation induced by inhalation of bacterial lipopolysaccharide. The kinetics of this DC response mimicked that of the neutrophil response, waxing and waning over a 48 h period, thus providing the immune system with an increased rate of "sampling" of antigens within the tissue milieu at the site of inflammation. Additionally, in experiments using an animal model of chronic airway inflammation induced by long-term exposure to pine dust (used as a model for cedar workers asthma), in which the airway epithelium expresses high levels of Ia and displays marked local eosinophilia, we have demonstrated that DC density (and Ia expression) increases to approximately double control levels [31]. Parenteral administration of recombinant γIFN to rats also increased DC density in both the airway and lung wall [77].

In humans, chronic inflammation induced by cigarette smoking is associated with surface phenotypic changes and numerical increases in lung and airway wall DCs, and increased frequency of intracytoplasmic Birbeck granules, the presence of which indicates DC activation [22]. DCs also contribute up to 1.1% of BAL cells in smokers but <0.1% in controls [78], and comparable DC influxes have been observed in rats in response to local inflammatory challenge in the deep lung (see discussion [57]).

An increased frequency of DCs has also been reported in the nasal mucosa of allergic rhinitis sufferers during the pollen season [79], associated with the presence of IgE on the surface of the cells. In view of the postulated role of Fc-receptor-bound IgE on DCs as an alternative pathway for allergen presentation to CD4$^+$T-cells at sites of allergic inflammation [80], the possible presence of this antibody on the surface of lung DCs warrants further investigation.

No information is currently available on the relative functional capacity of normal *versus* "inflammatory" DCs in the lung.

Mononuclear phagocytic cells (MPC)

The first evidence that inflammation induced major changes in regulation of T-cell activation in the lung stems from the original studies of MACKANESS [81] in the Listeria monocytogenes model in mice. He demonstrated that the induction of secondary T-cell mediated immunity in the deep lung could not occur until the resident PAM population had been diluted by a large influx of blood monocytes [81], and suggested that the latter provided a necessary source of active APCs to compensate for the functionally "defective"

PAMs. Our laboratory subsequently demonstrated that in addition to failing to present antigen to T-cells, resident PAMs actively suppressed T-cell proliferation and further showed that this suppression was reversed following the induction of a local inflammatory response which recruited fresh mononuclear cells for the blood [82]. Our original interpretation of these results, *viz.* that the active incoming cell was a monocyte, may require revision in the light of the demonstration by Havernith and Hoefsmit (see discussion in [57]) that DCs are also present in alveolar mononuclear infiltrates in this animal model.

Changes in macrophage populations have also been reported in the context of inflammatory diseases in the human lung. Active recruitment of monocytes into the airway mucosa is a consistent finding in asthma [83, 84], and in view of the known differences in cytokine production capacity of human monocytes compared to mature pulmonary macrophages, *e.g.* [85], such infiltration may contribute to the pathogenesis of the underlying inflammatory process. No functional data are yet available on macrophage function(s) in asthmatic *versus* normal airways but several reports exist on BAL. Reported findings from comparative studies of normal *versus* atopic BAL macrophages include equivalent levels of production of interleukin-1 (IL-1) inhibitory factor [86] and arachidonic acid metabolites [87], increased numbers of hypodense PAMs (suggestive of activation) in asthmatics [88], differing patterns of surface lectin-binding by asthmatic PAMs [89], depressed zymosan phagocytosis [90], and decreased *in vitro* T-cell inhibitory activity [91] by asthmatic PAMs. In addition, *in vitro* culture of PAMs with corticosteroids used for treatment of asthmatics (*via* inhalation) has been shown to modulate function-associated surface marker expression [92], but no formal data are yet available to indicate whether functional changes occur *in vitro* or *in vivo*.

More detailed information is available in pulmonary sarcoidosis. The salient feature of this syndrome is excess T-helper activity at sites of disease activity in the lung [93], which is accompanied by a number of surface phenotypic and functional changes in adjacent PAM populations. These include increased surface expression of class II MHC [94–96], enhanced capacity to present antigen to T-cells [97–99], and enhanced expression of surface antigens associated with the monocytic end of the MPC differentiation spectrum, consistent with increased recruitment of monocytes from the peripheral blood [100]. Consistent with this suggestion, sarcoid PAMs secrete high levels of IL-1 [101] analogous to monocytes, whereas normal PAMs are poor producers of this cytokine [85]. These findings have prompted the suggestion that alterations in the balance between inhibitory and stimulatory signals from the lung macrophage population may be an important aetiological factor in the enhanced local $CD4^+$ T-cell activity in this disease [100, 102].

It has also been reported that the APC activity of PAM populations can increase in certain types of infectious disease, notably tuberculosis [103], and human immunodeficiency virus (HIV) [104], but in neither case was this associated with increased IL-1 production as with sarcoidosis.

Other cell types

Increased expression of class II MHC antigens on mesenchymal cells in the lung is consistently reported throughout the literature on inflammatory disease. As noted above, this may represent a pathway for presentation of antigen-specific activating signals to T-cells, but the emerging literature on regulation of T-cell-receptor triggering points increasingly to the converse, *viz.* that activation by such Ia^+ "non-professional" APCs is incomplete, lacking the necessary (second) co-stimulator signal required for proliferation and clonal expansion, and consequently directs the T-cell down a maturation pathway towards T-cell-receptor downregulation and anergy. While this represents an attractive ancillary protective mechanism for dampening T-cell activity at inflammatory foci, formal proof of its operation *in vivo* is still lacking.

Little is also known of the role of the B-cell in T-cell activation in inflammatory diseases. B-cell infiltration is not a significant feature of the inflammatory process in the asthmatic airway wall [84], but traffic of these cells through inflammatory sites in other respiratory disease states could theoretically represent a source of antigenic signalling for adjacent draining lymph nodes. Again formal proof is lacking.

Conclusions

While there are clearly a variety of candidate APC populations for regulation of T-cell activation in different tissue microenvironments in the lung and conducting airways (summarized in table 1), an overall picture is slowly emerging of how the major cellular populations interact. Thus, under steady-state conditions, DC populations play a surveillance role for incoming antigens, shuttling them to the draining lymph nodes for eventual presentation to the T-cell system, and in disease free lung tissue they represent virtually the sole source of local APC activity. However, in response to inflammatory stimulation, the spectrum of local cell types expressing class II MHC is expanded *via* recruitment from the peripheral blood and *via* cytokine-driven "induction" of local mesenchymal cells, providing a range of optional APCs for upregulation and downregulation of local T-cell responses. The balance between these potential immunoregulatory APC populations are undoubtedly key factors in the pathogenesis of immunoinflammatory diseases throughout the respiratory system.

Table 1 – Potential antigen presenting cells in normal respiratory tract tissues

Properties	Dendritic cells	Mature macrophages	Monocytes	Bronchial epithelial cells	Type II alveolar epithelium	Fibroblasts	B-cells
Class II MHC expression							
Constitutive	++++	±	+	-	-	-	++
Cytokine-induced	++	+	++	+	+	+	+
Distribution							
Airway epithelium	+++*	-	-	+++	-	-	-
Airway submucosa	++	+++*	±*	-	-	+	±
Alveolar wall	++	+*	+*	-	++*	+	±
Airway lumen	±	+++*	±*	-	-	-	±
[Intravascular]	[+]	[+]	[+]	-	-	-	[+]
APC activity							
1° immune responses	++++	-	±	-	-	-	±
2° immune responses	++++**	-	++	[+]	-	[+]	++
Anergy/suppresion	-	++	-	[?]	[+]	[?]	-
Migratory capacity (to draining lymph nodes)	+++	+	[?]	-	-	-	+
Probable preferred antigen(s)	Soluble protein (allergens?) Viruses	Particulate (baterial)	Particulate (bacterial)	Soluble protein Viruses	Small particulates Soluble proteins	?	Soluble and Particulate

*: increased in inflammation; **: normally only within the regional lymph node; [+]: claim based on only a small number of observations, requiring verification; [?]: theoretically likely, but not presently known; MHC: major histocompatability complex; APC: antigen presenting cells.

References

1. Hamid Q, Azzawi M, Ying S, *et al.* – Expression of mRNA for IL-5 in mucosal bronchial biopsies from asthma. *J Clin Invest,* 1991; 87: 1541–1546.
2. Robinson DS, Hamid Q, Ying S, *et al.* – Predominant TH2-like bronchoalveolar T-cell population in atopic asthma. *N Engl J Med,* 1992; 326: 298–304.
3. Sedgwick JD, Holt PG. – Induction of IgE-secreting cells and IgE isotype-specific suppressor T-cells in the respiratory lymph nodes of rats in response to antigen inhalation. *Cell Immunol,* 1985; 94: 182–194.
4. Janeway CA, Bottomly K, *et al.* – Quantitative variation in Ia expression plays a central role in immune regulation. *Immunol Today,* 1984; 5: 99–105.
5. Lawerijns JM, Baert JH. – Alveolar clearance and the role of pulmonary lymphatics. *Am Rev Respir Dis,* 1977; 115: 625–648.
6. Tovey ER, Chapman MD, Platts-Mills TAE. – Mite faeces are a major source of house dust allergens. *Nature,* 1981; 289: 592–593.
7. Bretscher P. – The two-signal model of lymphocyte activation 21 years later. *Immunol Today,* 1992; 13: 74–76.
8. Janeway CA. – The immune response evolved to discriminate infectious nonself from noninfectious self. *Immunol Today,* 1992; 13: 11–16.
9. Steinman RM. – The dendritic cell system and its role in immunogenicity. *Ann Rev Immunol,* 1991; 9: 271–296.
10. Mueller DL, Jenkins MK, Schwartz RH. – Regulation of T-cell activation. *Ann Rev Immunol,* 1989; 7: 445–461.
11. Lassila O, Vainio O, Matzinger P. – Can B-cells turn on virgin T-cells? *Nature,* 1988; 334: 253–255.
12. Freudenthal P, Steinman RM. – Surface antigens and motility of human dendritic cells enriched by blood by an improved technique. *Proc Natl Acad Sci USA,* 1990; 87: 7698–7702.
13. MacPherson GG. – Lymphoid dendritic cells: their life history and roles in immune responses. *Res Immunol,* 1989; 140: 877–880.
14. Barfoot R, Denham S, Gyure LA, *et al.* – Some properties of dendritic macrophages from peripheral lymph. *Immunol,* 1989; 68: 233–239.
15. Kawamani O, Basset F, Ferrans VJ, Soler P, Crystal RG. – Pulmonary Langerhan's cells in patients with fibrotic lung disorders. *Lab Invest,* 1981; 44: 227–233.
16. Hamman S, Brockus D, Remmington S, Bartha M. – The widespread distribution of Langerhan's cells in pathological tissues. An ultrastructural and immunohistochemical study. *Hum Pathol,* 1986; 17: 894–905.
17. Richard S, Barbey S, Pfister A, *et al.* – Mise en evidence de cellules de Langerhan's dans l'epithelium bronchique humain. *CR Acad Sci Paris,* 1987; t.305 serie 111: 35–39.
18. Sertl K, Takemura T, Tschachler E, *et al.* – Dendritic cells with antigen-presenting capability reside in airway epithelium, lung parenchyma and visceral pleura. *J Exp Med* 1986; 163: 436–451.
19. Holt PG, Schon-Hegrad MA, Oliver J. – Localization of T-cells macrophages and dendritic cells in rat respiratory tract tissues: implications for immune function studies. *Immunol,* 1987; 62: 349–356.
20. 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: 262–274.
21. Holt PG, Degebrodt A, O'Leary C, Krska K, Plozza T.

– T-cell activation by antigen-presenting cells from lung tissue digests: suppression by endogenous macrophages. *Clin Exp Immunol*, 1985; 63: 586–594.
22. Soler P, Moreau A, Basset F, Hance AJ. – Cigarette smoking induced changes in the number and differentiated state of pulmonary dendritic cells/Langerhan's cells. *Am Rev Respir Dis*, 1989; 139: 1112–1117.
23. Nicod LP, Lipscomb MF, Weissler, JC, Toews GB. – Mononuclear cells from human lung parenchyma support antigen-induced T-lymphocyte proliferation. *J Leukocyte Biol*, 1989; 45: 336–344.
24. Nicod LP, Lipscomb MF, Weissler JC, *et al.* – Mononuclear cells in human lung parenchyma. Characterisation of a potent accessory cell not obtained by bronchoalveolar lavage. *Am Rev Respir Dis*, 1986; 136: 818–823.
25. Rochester CL, Goodell EM, Stoltenberg JK, Bowers WE. – Dendritic cells from rat lung are potent accessory cells. *Am Rev Respir Dis*, 1988; 138: 121–128.
26. Nicod LP, Lipscomb MF, Toews GB, Weissler JC. – Separation of potent and poorly functional human lung accessory cells based on autofluorescence. *J Leukocyte Biol*, 1989; 45: 458–465.
27. Holt PG, Oliver J, McMenamin C, Schon-Hegrad MA. – Studies on the surface phenotype and functions of dendritic cells in parenchymal lung tissue of the rat. *Immunol*, 1992; 75: 582–587.
28. Wolff K, Stingl G. – The Langerhan's cell. *J Invest Dermatol*, 1983; 80 (Suppl. 6): 17–21.
29. Holt PG, Schon-Hegrad MA, Oliver J, Holt BJ, McMenamin PG. – A contiguous network of dendritic antigen-presenting cells within the respiratory epithelium. *Int Arch Allergy Appl Immunol*, 1990; 91: 155–159.
30. Holt PG, Schon-Hegrad MA, Phillips MJ, McMenamin PG. – Ia-positive dendritic cells form a tightly meshed network within the human airway epithelium. *Clin Exp Allergy*, 1989; 19: 597–601.
31. Schon-Hegrad MA, Oliver J, McMenamin PG, Holt PG. – Studies on the density, distribution and surface phenotype of intraepithelial class II MHC (Ia) antigen-bearing dendritic cells in the conducting airways. *J Exp Med*, 1991; 173: 1345–1356.
32. Cumberbatch M, Kimble I. – Dermal TNFα induces dendritic cell migration to draining lymph nodes, and possibly provides one stimulus for Langerhan's cell migration. *Immunol*, 1992; 75: 257–263.
33. Schuler G, Steinman RM. – Murine epidermal Langerhan's cells mature into potent immunostimulatory dendritic cells *in vitro*. *J Exp Med*, 1985; 161: 526–532.
34. Reynolds HY. – Bronchoalveolar lavage. *Am Rev Respir Dis*, 1987; 135: 250–263.
35. Holt PG, Degebrodt A, Venaille T, *et al.* – Preparation of interstitial lung cells by enzymatic digestion of tissue slices: preliminary characterisation by morphology and performance in functional assays. *Immunol*, 1985; 54: 139–147.
36. Dethloff LA, Lehnert BE. – Pulmonary interstitial macrophages: isolation and flow cytometric comparisons with alveolar macrophages and blood monocytes. *J Leukocyte Biol*, 1988; 43: 80–90.
37. Holt PG. – Downregulation of immune responses in the lower respiratory tract: the role of alveolar macrophages. *Clin Exp Immunol*, 1986; 63: 261–270.
38. Holt PG, Warner LA, Papadimitriou JM. – Alveolar macrophages: functional heterogeneity within macrophage populations from rat lung. *Aust J Exp Biol Med Sci*, 1982; 60: 607–613.
39. Blusse van Oud Alblas A, Van Der Linden-Schrever B, van Furth R. – Origin and kinetics of pulmonary macrophages during an inflammatory reaction induced by intracellular administration of aerosolised heat-killed BCG. *Am Rev Respir Dis*, 1983; 128: 276–281.
40. Bitterman PB, Saltzman LE, Adelberg S, Ferrans VJ, Crystal RG. – Alveolar macrophage replication. One mechanism for the expansion of the mononuclear phagocyte population in the chronically inflamed lung. *J Clin Invest*, 1984; 74: 460–469.
41. Sawyer RT. – The cytokinetic behaviour of pulmonary alveolar macrophages in monocytopenic mice. *J Leukocyte Biol*, 1986; 39: 89–99.
42. Tarling JD, Lin H, Hsu S. – Self renewal of pulmonary alveolar macrophages. Evidence from radiation chimeral studies. *J Leukocyte Biol*, 1987; 42: 443–446.
43. Adamson IYR, Bowden DH. – Role of monocytes and interstitial cells in the generation of pulmonary alveolar macrophages. II. Kinetic studies after carbon loading. *Lab Invest*, 1980; 42: 518–524.
44. Holt PG. – Alveolar macrophages. II. Inhibition of lymphocyte proliferation by purified macrophages from rat lung. *Immunol*, 1979; 37: 429–436.
45. Warner LA, Holt PG, Mayrhofer G. – Alveolar macrophages. IV. Regulation of alveolar macrophage-mediated suppression of lymphocyte proliferation by a putative T-cell. *Immunol*, 1981; 42: 137–145.
46. Shellito J, Kaltreider HB. – Heterogeneity of immunologic function amongst subfraction of normal rat alveolar macrophages. *Am Rev Respir Dis*, 1984; 129: 747–753.
47. Murphy MA, Herscowitz HB. – Heterogeneity amongst alveolar macrophages in humoral and cell-mediated immune responses. *J Leukocyte Biol*, 1984; 35: 39–54.
48. Oghiso Y, Kubota Y. – Heterogeneity in immunologic functions amongst canine alveolar macrophages. *Jap J Vet Sci*, 1986; 48: 1125–1134.
49. Spiteri MA, Poulter LW. – Characterisation of immune inducer and suppressor macrophages from the normal human lung. *Clin Exp Immunol*, 1991; 83: 157–162.
50. Rich EA, Tweady DJ, Fujiwara H, Ellner JJ. – Spectrum of immunoregulatory functions and properties of human alveolar macrophages. *Am Rev Respir Dis*, 1987; 136: 258–265.
51. Harmsen AG, Muggenburg BH, Burton Snipes M, Bice DE. – The role of macrophages in particle translocation from lungs to lymph nodes. *Science*, 1985; 320: 1277–1280.
52. Thepen T, Claassen E, Hoeben K, Breve J, Kraal G. – Specific migration of alveolar macrophages from alveolar spaces to the paracortical T-cell areas of the draining lymph node. *Immunol*, (in press).
53. Thepen T, Van Rooijen N, Kraal G. – Alveolar macrophage elimination *in vivo is* associated with an increase in pulmonary immune response in mice. *J Exp Med*, 1989; 170: 499–509.
54. Thepen T, McMenamin C, Oliver J, Kraal G, Holt PG. – Regulation of immune response to inhaled antigen by alveolar macrophages (AM): differential effects of *in vivo* AM elimination on the induction of tolerance *versus* immunity *Eur J Immunol*, 1991; 21: 2845–2850.
55. Thepen T, McMenamin C, Girn B, Kraal G, Holt PG. – Regulation of IgE production in presensitised animals: *in vivo* elimination of alveolar macrophages preferentially increases IgE responses to inhaled allergen. *Clin Exp Allergy*, (in press).
56. Parra SC, Burnette R, Rice HP, Takaro T. – Zonal distribution of alveolar macrophages, type II pneumocytes, and alveolar septal connective tissue gaps in adult human lungs. *Am Rev Respir Dis*, 1986; 133: 908–912.

57. Holt PG, Schon-Hegrad MA, McMenamin PG. – Dendritic cells in the respiratory tract. *Intern Rev Immunol,* 1990; 6: 139–149.
58. Glanville AR, Tazelaar HD, Theodore J, *et al.* – The distribution of MHC class I and II antigens on bronchial epithelium. *Am Rev Respir Dis,* 1989: 139; 330–334.
59. Natali PC, De Martino C, Quarawta V. – Expression of Ia-like antigens in normal human non-lymphoid tissues. *Transplantation,* 1981; 31: 75–78.
60. Kalb TH, Chuang MT, Marom Zvi, Mayer L. – Evidence for accessory cell function by class II MHC antigen-bearing airway epithelial cells. *Am J Respir Cell Mol Biol,* 1991; 4: 320–329.
61. Ohtoshi T, Vancheri C, Cox G, *et al.* – Monocyte-macrophage differentiation induced by human upper airway epithelial cells. *Am J Respir Cell Mol Biol,* 1991; 4: 255–263.
62. Smith SM, Lee DKP, Lacy J, Coleman DC. – Rat tracheal epithelial cells produce GM-CSF. *Am J Respir Cell Mol Biol,* 1990; 2: 59–68.
63. Twomey JJ, Laughter A, Brown MF. – A comparison of the regulatory effects of human monocytes, PAM and spleen macrophages upon lymphocyte responses. *Clin Exp Immunol,* 1983; 52: 449–456.
64. Mezzetti M, Soloperto M, Fasoli A, Mattoli S. – Human bronchial epithelial cells modulate CD3 and mitogen-induced DNA synthesis in T-cells but function poorly as APC compared to pulmonary macrophages. *J Allergy Clin Immunol,* 1991; 87: 930–938.
65. Koyama S, Rennard SI, Leikauf GD, Robbins RA. – Bronchial epithelial cells release monocyte chemotactic activity in response to smoke and endotoxin. *J Immunol,* 1991; 147: 972–979.
66. Harbeck RJ, Gegen NW, Struhar D, Mason R. – Class II molecules on rat alveolar type II epithelial cells. *Cell Immunol,* 1988; 111: 139–147.
67. Paine R, Mody CH, Chavis A, *et al.* – Alveolar epithelial cells block lymphocyte proliferation *in vitro* without inhibiting activation. *Am J Respir Cell Mol Biol,* 1991; 5: 221–229.
68. Gaspari AA, Jenkins MK, Katz SI. – Class II MHC-bearing keratocytes induce antigen-specific unresponsiveness in hapten-specific TH-1 clones. *J Immunol,* 1988; 141: 2216–2220.
69. Phipps RP, Penney DP, Keng P, *et al.* – Characterisation of two major populations of lung fibroblasts: distinguishing morphology and discordant display of Thy 1 and Class II MHC. *Am J Respir Cell Mol Biol,* 1989; 1: 65–74.
70. Nelson D, Strickland D, Holt PG. – Selective attrition of non-recirculating T-cells during normal passage through the lung vascular bed. *Immunol,* 1990; 69: 476–481.
71. Holt PG, Kees U, Schon-Hegrad MA, *et al.* – Limiting dilution analysis of T-cells extracted from human lung tissue. *Immunol,* 1988; 64: 649–654.
72. Scheuermann DW. – Proliferation and transformation of lymphocytes in lung capillaries of the rat. *Acta Anat,* 1982; 13: 264–280.
73. Beacham CH, Daniele RP. – Migration of recently divided B and T lymphocytes to peritoneum and lung. *Cell Immunol,* 1982; 74: 284–293.
74. Van Furth R, Sluiter W. – Distribution of blood monocytes between a marginating and a circulating pool. *J Exp Med,* 1986; 163: 474–479.
75. Winkler GC. – Pulmonary intravascular macrophages in domestic animal species: review of structural and functional properties. *Am J Anat,* 1988; 181: 217–234.
76. Dehring DJ, Wismar BL. – Intravascular macrophages in pulmonary capillaries of humans. *Am Rev Respir Dis,* 1989; 139: 1027–1029.
77. Kradin RL, McCarthy KM, Xia Weijin, Lazarus D, Schneeberger EE. – Accessory cells of the lung. I. Interferon-gamma increases Ia$^+$ dendritic cells in the lung without augmenting their accessory activities. *Am J Respir Cell Mol Biol,* 1991; 4: 210–218.
78. Casolaro MA, Bernaudin J-F, Soltini C, Ferrans VJ, Crystal RG. – Accumulation of Langerhan's cells on the epithelial surface of the lower respiratory tract in normal subjects in association with cigarette smoking. *Am Rev Respir Dis,* 1988; 137: 406–411.
79. Fokkens WJ, Vroom TMA, Rijnijes E, Mulder PGH. – CD1$^+$ (T6) HLA-DR-expressing cells, presumably Langerhan's cells in nasal mucosa. *Allergy,* 1989; 44: 167–172.
80. Fokkens WJ, Bruijnzeel-Koomen CAFM, Vroom TM, *et al.* – The Langerhan's cell: an underestimated cell in atopic disease. *Clin Exp Allergy,* 1990; 20: 627–638.
81. Mackanness GB. – The induction and expression of cell-mediated hypersensitivity in the lung. *Am Rev Respir Dis,* 1971; 104: 813–826.
82. Holt PG. – Inhibitory activity of unstimulated alveolar macrophages on T-lymphocyte blastogenic responses. *Am Rev Respir Dis,* 1978; 118: 791–793.
83. Beasley R, Roche WR, Roberts JA, Holgate ST. – Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis,* 1989; 139: 806–817.
84. Poston RN, Chanez P, Lacoste JY, *et al.* – Immunohistochemical characterisation of the cellular infiltration in asthmatic bronchi. *Am Rev Respir Dis,* 1992; 145: (in press).
85. Wewers MD, Rennard SI, Hance AJ, Bitterman PB, Crystal RG. – Normal human alveolar macrophages obtained by BAL have a limited capacity to release IL-1. *J Clin Invest,* 1984; 74: 2208–2215.
86. Gossett O, Lassalle P, Tonnel AB, *et al.* – Production of an IL-1 inhibitory factor by human alveolar macrophages from normals and allergic asthmatic patients. *Am Rev Respir Dis,* 1988; 138: 40–46.
87. Balter MS, Eschenbacher WL, Peters-Golden M. – Arachidonic acid metabolism in cultured alveolar macrophages from normals and allergic asthmatic patients. *Am Rev Respir Dis,* 1988; 138: 1134–1142.
88. Chanez P, Bousquet J, Couret L, *et al.* – Increased number of hypodense alveolar macrophages in patients with bronchial asthma. *Am Rev Respir Dis,* 1991; 144: 923–930.
89. Warton A, Phillips MJ, Papadimitriou JM. – Ultrastructural and cytophotometric studies of lectin-binding by human PAM from asthmatic and normal subjects. *Aust J Exp Biol Med Sci,* 1986; 64: 419–434.
90. Godard P, Chaintreuil J, Damon M, *et al.* – Functional assessment of alveolar macrophages: comparison of cells from asthmatics and normal subjects. *J Allergy Clin Immunol,* 1982; 70: 88–93.
91. Aubas P, Cosso 13, Godard P, Michel FB, Clot J. – Decreased suppressor cell activity of alveolar macrophages in bronchial asthma. *Am Rev Respir Dis,* 1984; 130: 875–878.
92. Marmayagam L, Poulter LW. – Corticosteroid can alter antigen expression on alveolar macrophages. *Clin Exp Immunol,* 1991; 85: 531–535.
93. Gallagher RB, Guckian M, van Breda A, *et al.* –

Altered immunological reactivity in alveolar macrophages from patients with sarcoidosis. *Eur Respir J*, 1988; 1: 153–160.
94. Campbell DA, du Bois RM, Butcher RG, Poulter LW. – The density of HLA-DR antigen expression on alveolar macrophages is increased in pulmonary sarcoidosis. *Clin Exp Immunol*, 1986; 65: 165–171.
95. Spurzen JR, Saltini C, Kirby M, Konishi K, Crystal RG. – Expression of HLA Class II genes in alveolar macrophages of patients with sarcoidosis. *Am Rev Respir Dis*, 1989; 140: 89–94.
96. Haslam PL, Parker DJ, Townsend PJ. – Increases in HLA-DQ, DP, DR, and tranferrin receptors on PAM in sarcoidosis and allergic alveolitis. *Chest*, 1990; 97: 651–661.
97. Lem VM, Lipscomb MF, Weissler JC, *et al.* – BAL cells from sarcoid demonstrate enhanced antigen presentation. *J Immunol*, 1985; 135: 1766–1771.
98. Venet A, Hance AJ, Saltini C, Robinson BWS, Crystal RG. – Enhanced PAM-mediated antigen-induced T-cell proliferation in sarcoidosis. *J Clin Invest*, 1985; 75: 298–301.
99. Ina Y, Yamamoto M, Takada K, *et al.* – Antigen presenting capacity in sarcoidosis. *Am Rev Respir Dis*, 1988; 137: 341.
100. Hance AJ, Douches S, Winchester RJ, Ferrans VJ, Crystal RG. – Characterisation of mononuclear phagocyte subpopulations in the human lung by using monoclonal antibodies: changes in alveolar macrophage phenotype associated with pulmonary sarcoidosis. *J Immunol*, 1985; 134: 284–292.
101. Hunninghake GW. – Release of IL-1 by PAM of patients with active pulmonary sarcoidosis. *Am Rev Respir Dis*, 1984; 129: 569–572.
102. Poulter LW. – Changes in lung macrophages during disease. *FEMS Microbiol Immunol*, 1990; 64: 327–332.
103. Ina Y, Takada K, Yamamoto M, Morishita M, Yoshikawa K. – Antigen presenting capacity of PAM and monocytes in pulmonary tuberculosis. *Eur Resp J*, 1991; 4: 88–93.
104. Twigg HL, Lipscomb MF, Yoffe B, Barabaro DJ, Weissler JC. – Enhanced accessory cell function by PAM from patients infected with HIV: potential role for depletion of $CD4^+$ cells in the lung. *Am J Respir Cell Mol Biol*, 1989; 1: 391–400.