Review

Immune homeostasis in the respiratory tract and its impact on heterologous infection

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1. Introduction

An immune response ensues when a pathogen or traumatic event is sufficiently dangerous that it exceeds a specific threshold of innate restraint. The restraint of innate (and ultimately adaptive) immunity occurs throughout the entire body, but the threshold of reactivity is adjusted in a site-specific manner [1,2]. Certain body compartments are bombarded with foreign material and must ignore the majority of it (the gut and lung), whereas others are essentially sterile and tend to respond immediately (the blood and spleen). Lung alveolar macrophages, for example, exist in an environment high in antigenic material, the majority of which must be ignored except when the antigen represents a serious threat [3,4]. Even the ignorance of antigen requires that the environment is sampled to ensure that particles present should indeed be ignored. For example, M cells [5,6] in the gut and parts of the lung sample the luminal contents for signs of a threat. Sub-mucosal immune cells are present and poised to react should they be required. Lung dendritic cells extend processes into the airway lumen to scan, interact with and, potentially draw antigens towards the waiting sub-mucosal immune population [7,8].

This site-specific immune response and the status of innate immunity in homeostasis however need to be adaptable. It would not make evolutionary sense for our threshold of reactivity (above which innate immunity activates) to be set before or immediately after birth and remain in the same state throughout life. It would also possibly not make sense for innate immunity to return to the exact same state after resolution of a serious inflammatory event. Innate homeostasis may therefore be adaptable; altered by environmental influences, genetics, diet, stress, age and the sequence of prior inflammatory events. This adaptability will contribute to the diversity of responses to the same stimulus seen in any given population and probably helps to ensure that at least a proportion of those affected will survive. If thresholds of innate activation alter between body compartments and with each inflammatory event, what pathways are involved? What dictates whether a macrophage in a particular environment responds readily to antigen or essentially tolerates its presence? A tissue-specific switch must exist that is made up of a unique signature composed of immune regulatory versus immune potentiator pathways. This phenomenon of innate homeostasis and adaptability will be described for the lung microenvironment, in particular the airways. We will highlight similar processes in other sites where data is available and describe the consequences for subsequent immune reactions to respiratory pathogens.

2. The airways in homeostasis: innate immunity

The healthy airways in man and mouse are dominated by alveolar macrophages (>95%). The remaining 5% are predominantly T cells expressing CD103+, γδ or αβ TCR and are generally CD4-/CD8- (or combinations of these phenotypes). Though alveolar macrophages express typical phenotypic markers (F4/80+, CD11c+, CD11bint), functionally they behave differently to their tissue counterparts, even compared to those in the sub-mucosal tissues of the lung parenchyma. For example, alveolar macrophages are thought to be less efficient as antigen-presenting cells, with
their tissue-resident counterparts [9]. In the steady-state they display a suppressive phenotype mediated by the secretion of IL-10, nitric oxide or TGFβ [10,11]. They are also poorly phagocytic compared to peritoneal macrophages [12] and actively inhibit T cell responses via secretion of prostaglandins and transforming growth factor beta (TGFβ) [13]. Alveolar macrophages produce a state of reversible T cell inactivation that is mediated either by defective expression of co-stimulatory molecules [14], or an increased expression of CD80 [15], a receptor that preferentially binds to the negative T cell receptor CTLA-4. The direct or indirect interaction of alveolar macrophages with other cells in the airways may also limit inflammatory responses in the steady-state. In addition to the suppression of T cell activation described above, alveolar macrophages also actively inhibit the antigen presentation function of interdigitating dendritic cells in the airways [16]. Reciprocally, the DCs exposed to antigen in the airway lumen produce IL-10, a broadly suppressive phenotype mediated by the secretion of IL-10, TGFβ and IL-4 [17]. This cytokine serves as a paracrine mediator of airway epithelial cell and alveolar macrophage TLR 2–5, 7 and 9 responses via secretion of prostaglandins and transforming growth factor beta (TGFβ) [13]. Alveolar macrophages produce a state of reversible T cell inactivation that is mediated either by defective expression of co-stimulatory molecules [14], or an increased expression of CD80 [15], a receptor that preferentially binds to the negative T cell receptor CTLA-4. The direct or indirect interaction of alveolar macrophages with other cells in the airways may also limit inflammatory responses in the steady-state. In addition to the suppression of T cell activation described above, alveolar macrophages also actively inhibit the antigen presentation function of interdigitating dendritic cells in the airways [16]. Reciprocally, the DCs exposed to antigen in the airway lumen produce IL-10, a broadly inhibitory cytokine which further limits the local inflammatory response [17].

Macrophages in the airways are functionally different to phenotypically identical counterparts elsewhere by several other suppressive pathways that in combination limit their responsiveness to external stimuli. Surfactants, which are proteins and lipids that are abundant in the alveolar spaces where they function to decrease surface tension at the air–fluid interface, allowing the airspaces to remain open [18]. Surfactant protein A (SP-A) has recently been shown to inhibit downstream signalling via Akt from TLR2 and TLR4, and as a result decreases pro-inflammatory cytokine production from stimulated human airway macrophages [19]. SP-A also binds to TLR4 and thus blocks binding of LPS to the receptor and subsequent downstream activation signals [20]. Furthermore, SP-A, along with a related family member SP-D, reduces the phagocytic activity of alveolar macrophages by binding to and activating the signal inhibitory regulatory protein alpha (SIRPa), an inhibitory receptor highly expressed on these cells [12]. MUC1, a transmembrane mucin-like glycoprotein expressed on epithelial cells, suppresses mouse airway epithelial cell and alveolar macrophage TLR 2–5, 7 and 9 signalling pathways, suggesting yet another anti-inflammatory strategy [21]. This plethora of negative regulators maintains airway luminal cells in a quiescent state during homeostasis in a unique environmental niche that is unlikely to be found elsewhere.

We have recently identified another novel homeostatic loop mediated by CD200 receptor (CD200R), which is required for homeostasis and resolution of lung myeloid cell activity. CD200 receptor is almost exclusively expressed by myeloid cells, including macrophages and dendritic cells [22–26]. CD200 ligand however is expressed by a variety of cells such as thymocytes, B cells, some peripheral T cells, neurons in the central nervous system and endothelium [27–32]. Binding of CD200 to CD200R imparts a unidirectional negative signal to CD200R bearing cells [22,25–27,33]. All of the cells expressing CD200 ligand can therefore potentially negatively regulate innate myeloid responses. The precise molecular mechanisms leading to suppression via CD200R signalling is not currently known. CD200R lacks ITIM domains commonly found in immune inhibiting receptors that cause recruitment of phosphatases and down regulation of inflammatory pathways. The cytoplasmic tail is 67 amino acids in length and contains 3 tyrosine residues as potential phosphorylation sites. One of these tyrosine residues is located within a NPXY motif, which is phosphorylated upon ligation of the receptor. This leads to the recruitment of Dok-1 and Doc-2, which are in turn phosphorylated and associate with RasGAP and SHIP. In mast cells it is known that this then inhibits the phosphorylation of ERK, P38 and JNK [24].

Alveolar macrophages, unlike counterparts in any other site, express unusually high basal levels of CD200R. Its ligand, CD200, is only expressed on the luminal aspect of the airway epithelium during homeostasis and in vitro studies show that this interaction prevents activation of alveolar macrophages in the presence or absence of inflammatory stimuli. Low levels of CD200R on splenic macrophages can be increased by incubation with IL-10 and TGFβ. TGFβ is abundant in the lung and present on respiratory epithelial cells tethered to the integrin α6β4 [34,35]. This together with high levels of IL-10 [36] are likely to increase CD200R on any macrophage trans-migrating into the airspaces and effectively sets up a tissue-specific microenvironment that governs the reactivity of innate immunity. Just taking into account CD200R, TGFβ and IL-10 a picture emerges clearly implying site-specific immunity. We have combined such pathways, and their balance by innate immune potentiators (for example, Toll-like receptors) into a concept called the innate immune rheostat. Rheostats are more commonly found in electrical appliances and a good example is the one found in a dimmer switch for a light bulb. This variable rheostat can be adjusted to dim or brighten the light. Similarly, negative and positive immune pathways form a variable “innate immune rheostat”, which dictates the threshold above which a particle is deemed a threatening antigen leading to an inflammatory response. In the resting airflow the alveolar macrophage “innate immune rheostat” is poised centrally, not too dim and not too bright (Fig. 1A).

This concept is also highly relevant to immunity in the gastrointestinal tract. Once again IL-10 is abundant from macrophages [37,38] and possibly regulatory T cells [39] and TGFβ is provided by the epithelium [40]. The gut is also abundant in thymic stromal lymphopoietin (TSLP), retinoic acid and vasoactive intestinal peptide that all down-regulate innate immune responses and provide an intestinal specific inhibitory signature (for a review see [41]). Similar to the events likely to occur on migration of macrophages into the airspaces, blood derived monocytes entering the gut will be conditioned by this controlled environment.

3. The airways in homeostasis: immune potentiators

What of immune potentiators in the lung during homeostasis? The activity of these is likely to overcome the homeostatic pathways that limit inflammation described above. Once again, site-specific regulation of their activity, distribution and abundance must differ between body compartments. Pattern recognition receptors, the most studied of which are the Toll-like receptors [42], recognise pathogens associated molecular patterns on pathogenic, but also commensal, microorganisms [43]. Their wide distribution on dendritic cells, monocyte/macrophages and epithelial cells [44–46] would make non-sterile mucosal tissues a highly inflammatory environment were it not for their altered threshold of responsiveness [46–49], decreased expression [45,50] and altered distribution [41,51,52] (for a review see [41]). In the intestine there is even evidence to suggest that TLRs prevent inflammation [53] by increasing epithelial integrity [54] and promoting B cell responses and IgA production [55]. In the colon suppression of TLR responses is mediated by an increase of negative regulators such as IRAK-M [56], SIGIRR [57] and Tollip [58]. In the lung however, much less is known about the expression and particularly the regulation of immune potentiators such as TLRs on airway macrophages and the vast epithelial surface. TLRs are clearly expressed on human bronchial epithelial cells and can respond to their agonists [47,59] (for a review see [47,60,61]). However, like the gut epithelium expression may be limited by site-specific regulatory mechanisms [47,62]. Other pattern recognition receptors are also present throughout the respiratory tree epithelium including the trachea [47]. TLR4 and the accessory molecule MD-2 are constitutively expressed in human alveolar and bronchial epithelial cells, though flow cytometry and confocal microscopy reveal them to be intracellular rather than displayed on the surface. Once again
this implies a site-specific strategy for limiting immediate inflammation [63], though pathways affecting their localisation are not currently known. TLR expression on airway macrophages is less well understood, parenchymal counterparts appear to respond in a similar manner to those in other "sterile" environments, whereas airway macrophages are altered, in their responsiveness at least, by airway specific factors such as surfactant proteins [19,20] and possibly cell surface receptors such as CD44 that limit responsiveness of murine alveolar macrophages via the intracellular negative regulators IRAK-M, Tollip and A20 [64]. Human alveolar macrophages in homeostasis express TLR2 [65] and though LPS stimulation up-expressed by epithelial produced IL-10 [36]. Intestinal epithelial cells are usually refractory to TLR4 signalling that is overcome by exposure to IFNγ and perhaps signalling via other TLRs [70]. IFNγ and TNF up-regulate TLR2 in respiratory epithelial cells [71] that is usually suppressed by promoter hypomethylation [72]. The inhibition of T cells responses by alveolar macrophages is overcome by the addition of pro-inflammatory cytokines such as GM-CSF and TNFα [73,74]. Once activated alveolar macrophages generally display a greater phagocytic capacity [75] with a higher oxidative burst and pro-inflammatory cytokine production [9], keeping with their role as a first line of defence in the respiratory tract.

A number of pathogens in the lung and gut adhere to, or directly infect, the mucosal epithelium. Lung epithelial cells upregulate Toll-like receptors during respiratory syncytial virus and influenza infection, which may be mediated by the release of IFN by infected macrophages [62,76,77]. This would result in the liberation of inflammatory mediators from a vast surface area. Furthermore, within the first 4 h of *Pseudomonas aeruginosa* infection NFκB activity is mainly detected within the bronchial epithelium [78]. Inflammation and pathogen replication may also result in the breakdown of epithelial integrity. A continuous epithelial barrier is vital to physically exclude exogenous organisms from the body's interior. Epithelial disruption doesn't just open the gates to within but also allows organisms access to macrophages and other potent antigen presenting cells that may be much less regulated than luminal counterparts. This is especially true in the respiratory tract.

4. Overcoming innate homeostasis and adjusting the innate immune rheostat towards inflammation

In both the lung and gut the tight control of innate immunity must be overcome in order for inflammation to proceed. In both sites it is likely that epithelial integrity is paramount and once this is inflamed or breached site-specific homeostasis is lost. Though pattern recognition receptors have an altered distribution and responsiveness in mucosal tissues these too will be adjusted in an inflamed environment or the threshold required to activate cells expressing them exceeded. Signalling via one TLR in alveolar macrophages profoundly changes the pattern of expression of others [66,68]. Exposure of alveolar macrophages to oxidative stress enhances exocytosis and hence surface expression of TLR4, that can be reversed with anti-oxidants [69]. Stimulation of alveolar macrophages through TLR2, TLR4, or TLR9 inhibits IL-10R signal transduction and releases them from the suppression usually mediated by epithelial produced IL-10 [36]. Intestinal epithelial cells are usually refractory to TLR4 signalling that is overcome by exposure to IFNγ and perhaps signalling via other TLRs [70]. IFNγ and TNF up-regulate TLR2 in respiratory epithelial cells [71] that is usually suppressed by promoter hypomethylation [72]. The inhibition of T cells responses by alveolar macrophages is overcome by the addition of pro-inflammatory cytokines such as GM-CSF and TNFα [73,74]. Once activated alveolar macrophages generally display a greater phagocytic capacity [75] with a higher oxidative burst and pro-inflammatory cytokine production [9], keeping with their role as a first line of defence in the respiratory tract.

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Airway macrophages are suppressed by IL-10, TGFβ and CD200R (and a plethora of other molecules), whereas tissue macrophages are less so. A sub-mucosal macrophage response to an organism is therefore likely to be qualitatively and quantitatively different.

Furthermore, disruption of the epithelium causes the loss of many regulatory pathways. Toll-like receptor signalling causes a down regulation of the αβ integrin that tethers TGFβ to the epithelial surface in the lung [34]. Epithelial expression of CD200 is also reduced, releasing the airway macrophage from inhibition via CD200R. A lack of signals through CD200R results in higher inflammatory cytokine, chemokine and reactive metabolite release from macrophages [79]. Blood derived monocytes and also adaptive immune cells entering this site are thus conditioned to be inflammatory as the calming environment has been lost. Inflammation often perpetuates inflammation through autocrine and paracrine pathways. The innate immune rheostat is thus firmly set to the inflammatory position (Fig. 1B).

5. Return to homeostasis

How then does peace return, and does the environment regain its original homeostatic state? It can often take more effort (by the same pathways) to return to homeostasis after inflammation than to maintain it in the first place. Homeostatic pathways prevent inflammation in the first instance, limit its magnitude once initiated and mediate resolution. However, the importance and activity required of homeostatic pathways will vary at each of these stages. For example, CD200R is high on resting alveolar macrophages compared to tissue-resident counterparts. On resolution of influenza induced inflammation however at day 14, CD200R intensity (determined by the geometric mean of fluorescence by flow cytometry) is exponentially higher on airway macrophages, and remains so for some time [79]. It is possible that this represent an overshoot in mechanisms trying to regain control of innate immunity in the airspaces. A similar case could be made for IL-10. In homeostasis IL-10 contributes to maintain a peaceful microenvironment. During influenza infection however, lymphocytes and macrophages are recruited that can also produce IL-10 [80]. CD11c expressing cells capable of producing IL-10 are maintained in the lung and display enhanced antigen presentation long after the resolution of an influenza or RSV primary infection [81,82]. Furthermore DCs that remain in the lung several weeks after infection have a more activated phenotype [81] and may also contribute to prolonged IL-10 concentrations in the airspaces. In influenza recovered mice a 50-fold increase in IL-10 is observed that depletion studies suggest is responsible for subsequent susceptibility to secondary bacterial colonisation in the lung [83].

TGFβ is secreted by virtually all cells as a biologically inactive protein termed latent TGFβ [84]. As TGFβ receptors are widely and constitutively expressed, regulation of this pathway is mediated dominantly by the balance between the latent and active form of this cytokine. In vitro studies show that influenza viral neuraminidase (NA) directly activates latent TGFβ, possibly through cleavage of the sialic acid residues on the amino-terminal latency-associated peptide (LAP) that remains non-covalently associated with the carboxy-terminal mature TGFβ molecule [85]. Though influenza infection may increase TGFβ levels during active infection of the lung resolved homeostatic levels have not yet been ascertained.

The up-regulation of IL-10 and CD200R levels alone long after influenza virus has been cleared would reset the innate immune rheostat below its prior homeostatic level. In addition, however, we are also faced with a long term alteration of immune potentiator pathways. Our recent study suggests that TLR responsiveness itself in alveolar macrophages (and possibly respiratory epithelial and dendritic cells) is blunted for long periods of time after influenza has been cleared from the lung [86]. This long term direct or indirect de-sensitisation of lung alveolar macrophages to the TLR ligands Flagellin, LPS and lipoteichoic acid (LTA) culminates in impaired NF-kB nuclear translocation, inflammatory cytokine production and cellular recruitment. This phenomenon is not simple TLR cross-tolerance, whereby TLR-activated cells are, for a short period of time, refractory to subsequent TLR stimulation [87] as the effect persists for months after the initial infection. Taking into account the slow turn over of airway macrophages [88], it is clear that the innate responsiveness of sentinel cells in the airway is molded by prior infection history.

This subtle alteration of responsiveness with time and after each wave of infection is not unique to the lung. In the gut, the microbial flora plays an essential role in mucosal homeostasis by actively inhibiting intestinal innate responses (for example see [89]). Furthermore, responsiveness of intestinal epithelial cells to TLR activation is impaired immediately after birth by exposure to exogenous endotoxin [90].

Inflammatory resolution therefore produces a different type of status quo (see Fig. 1C). In the second half of this review we will examine some of the consequences of altering the innate immune rheostat.

6. The consequences of an altered homeostatic state in the lung after inflammation has subsided

It is well established that two infections lead to a different pathological outcome compared to each alone. The lung microenvironment and its homeostatic profile can be altered considerably by prior events that leave behind long term consequences. These prior events may not be infectious. Emphysema, for example, enhances TLR2 and TLR4 leading to enhanced inflammation during Streptococcus pneumoniae pulmonary infection [91], whereas morphine reduces TLR9 induced NF-kB signalling and impairs S. pneumoniae clearance [92]. Cigarette smoke and COPD decrease TLR2 expression on alveolar macrophages potentially leaving the host susceptible to respiratory bacteria [93,94]. Ethanol and burn injury lead to a marked cellular infiltration in the lung and gut, higher serum inflammatory cytokines and a poor prognosis after pulmonary pseudomonas infection [95]. In the case of burn injury the alterations in a distant organ such as the lung is thought to arise from gut-derived factors carried in the mesenteric lymph that alter lung vascular permeability [96]. Finally, individuals may have underlying genetic alterations [97,98] that affect homeostasis and the ability to limit commensal organisms. C57/HEJ mice, for example, have increased terminal airspaces, three times more alveolar macrophages and enhanced matrix metalloproteinase 12, MCP-1 and KC, but also enhanced recoverable bacteria from the lungs compared to other mouse strains [99]. It is likely that polymorphisms in the regulatory or immune potentiator pathways described previously will also impact on immune homeostasis in the lung leading to altered responsiveness to the same pathogen within a population. In the remainder of this review however, we will focus on how lung infections alter the outcome for subsequent respiratory pathogens.

7. Innate imprinting and susceptibility to infection

The lungs response to infection will depend on each individual’s position on the innate immune rheostat. Infection of the lung in the absence of prior infection will produce a different outcome to the same pathogen infecting a lung with resolved inflammation. This alteration is not restricted to latent or concurrent infections but can also be influenced by acute inflammatory lung disease. Epidemiological studies show that influenza vaccination is associated with reduced susceptibility to subsequent, and indeed less severe, infections with Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis [100]. The innate imprints of the lung microenvironment made by recent infection may also play a role in the development of bacterial resistance to infection. For example, coinfection of mice with influenza virus and Staphylococcus aureus increases susceptibility to reinfection with an otherwise avirulent strain of S. aureus 36h post-infection, while the presence of S. aureus from birth results in prolonged persistence of S. pneumoniae in the nasopharynx [101].
logical and animal model data suggest that influences from prior unrelated pathogens may be long lasting [100–102]. An acute pulmonary influenza infection, for example, alters immunopathology to respiratory syncytial virus and heightened clearance of vaccinia virus is evident in lymphocytic choriomeningitis virus (LCMV) immune mice [102–104]. The outcome however is not always beneficial and may depend on the precise sequence of infection. Though influenza inhibits vaccinia virus replication it enhances the titres of LCMV and MCMV [104]. In addition to the de-sensitisation of the lung microenvironment to TLR ligands by influenza described above [86], some cause a skewing of the final cytokine profile of T cells responding to a second unrelated lung pathogen. For example, lung BCG infection improves the outcome during infection with the fungal pathogen Cryptococcus neoformans. In C57BL/6 mice C. neoformans induces a non-protective Th2 response in the lungs culminating in eosinophilic lung disease and systemic spread of the organism [105]. Prior BCG infection however skews the immune towards a protective Th1 response [106]. This is similar to the influence of Th1 inducing infections or antigens on the development of allergy and the subsequent rise in allergy due to a clean environment—the hygiene hypothesis (reviewed elsewhere [107]).

Even the transient immunity evoked by pathogen products can have an impact on heterologous immunity. Microbial products such as CpG DNA or a modified bacterial labile toxin (LTbK3) afford protection against a variety of subsequent respiratory pathogens [108,109]; some by altering a Th2 to a Th1 cytokine response [108] and others by maturation of antigen presenting cells in the lung microenvironment [109]. Microarray analysis shows that the modified E. coli endotoxin, LTKb3, induces a transient innate and adaptive immune response by itself [110], but long after these effects have subsided it reduces inflammation to a subsequent RSV and influenza pulmonary infection [109]. This alteration does not impact on pathogen clearance, which in the case of influenza is actually improved. This phenomenon in a number of cases does not involve cross-reactive T and B cells and is evident even in LTKb3 treated RAG knock out mice. Prior infection or particulate stimulation thus alters the innate immune rheostat making the cells involved behave in a qualitatively and quantitatively different manner. It is likely that other environmental particulates adjust the lung microenvironment in a similar manner as we alluded to above, though studies in this area are only beginning to appear.

Even events in the gut impact on lung immunity. Colonic restricted Citrobacter rodentium infection modulates the usual Th2 driven pathology to lung C. neoformans infection by skewing the immune response towards a Th1 cytokine profile [111]. Intrapitoneal injection of LPS into rats that have had their mesenteric lymph duct ligated attenuates lung injury, lung permeability, and PMN CD11b expression [112], suggesting gut-derived soluble factors or migrating cells influence distant organs or that gut/mesenteric lymph duct derived products (such as lipopolysaccharides) usually blunt pulmonary responses in health [113]. The longer term impact of gut inflammation on lung immune homeostasis and response to infection however has not been studied. The impact on the lung by spill over of inflammatory cells and cytokines from distant sites has been examined with regards to more persistent infection. It is important to note however, that the impact on the lung innate immune rheostat (in the absence of additional lung infection) is not known; i.e. what do chronic infections do to the lung per se? Helminths provide a clear example of a prolonged infection that can alter immune reactivity elsewhere. They generally induce excessive Th2 cytokine dominated immunity that could impact on Th1 associated respiratory infections [114–116]. Similarly, the induction of regulatory T cells to one infection may impact on others in a bystander fashion. Mycobacteria, malaria and parasitic protozoa and helminths induce high levels of immune suppressive cytokines (TGFβ and IL-10) that could reduce immunity to subsequent or concurrent infection in the respiratory tract. Vaccines proven to be efficient in high income countries might perform less well in low income countries [117] for the very same reasons.

Lung infections can also have an impact on other sites. Pulmonary murine gamma herpes virus latency affects peritoneal macrophages for up to 2 months with increased activation and expression of MHC class II. Latent herpes virus infection affords resistance to subsequent intranasal or intraperitoneal infection with Listeria monocytogenes or Yersinia pestis [118] and modulates the host inflammatory responses and susceptibility to mouse adenovirus type 1 [119]. Similar to our observations [86,102] this protection is not due to cross-reactive antigens but involves prolonged production of the anti-viral cytokines, systemic activation of macrophages and an alteration of basal innate immunity. Based on prior events both within and external to the lung we are therefore all unique with respect to responsiveness to respiratory infection [120].

8. Innate imprinting and secondary bacterial pneumonia

Another significant consequence of lung infection, influenza in particular, is the pre-disposition to secondary bacterial pneumonia. This is often regarded as a complication during influenza induced damage. However the influence of respiratory viral infection can actually be long lasting leaving the host susceptible to bacterial pneumonia long after the virus has been cleared [86]. Once again, the outcome of lung bacterial infection will depend on the position of the innate immune rheostat. Influenza causes significant and well publicised mortality as a single infection, but in combination with a secondary bacterial pneumonia is far more severe [121]. With a bacterial co-infection it ranks the 7th leading cause of mortality (5th in children) in the United States alone [122]. Amongst the 40–50 million deaths during the 1918–1919 influenza pandemic a large proportion had co-existing bacterial pneumonia ([123] and references therein). Re-cut autopsy tissue specimens taken from those that died in the 1918–1919, and also the 1957 and 1968, pandemic showed a high frequency of a variety of different bacterial organisms in those infected with influenza, and in some cases multiple bacterial species were present [123]. A wealth of clinical and animal model evidence proves that combined infections are worse than each alone. Fatalities in the 1957 influenza pandemic were higher in those with a co-existing respiratory bacterium [124]. Furthermore, seasonal influenza vaccination reduces mortality to combined bacterial infection [125] and anti-virals that target the viral neuraminidase prevent or slow respiratory bacterial infection in influenza infected mice [126,127].

Secondary bacterial complications are not a feature of influenza virus alone. Rhinovirus, respiratory syncytial virus and enteroviruses also induce viral wheezing that is invariably associated with bacterial infection leading to acute otitis media, sinusitis, interstitial pneumonia and, less frequently, alveolar pneumonia [128]. Furthermore, the low risk of concurrent bacterial infection is enhanced in pre-term infants hospitalised with RSV. Co-infection doubles the mean length of stay and bacterial isolates from those in the intensive care unit include S. pneumoniae, C. pneumoniae and a combination of S. pneumoniae with Haemophilus influenzae [129]. Analysis of children admitted to paediatric intensive care for RSV infection during three consecutive seasons reveals that 40% of them have a secondary bacterium in their lower airways [130]. This effect can be reproduced in mice where RSV infection decreases bacterial clearance due to functional alteration of neutrophils [131].

The reasons for enhanced susceptibility to bacterial pneumonia during influenza infection have been studied extensively (see Fig. 2) but the pre-disposition caused by other respiratory infections is less well known. Influenza is a cytopathic (lytic) virus...
Fig. 2. The consequences of influenza infection that lead to secondary bacterial pneumonia. The airways in homeostasis are dominated by airway macrophages that are suppressed by site-specific regulatory pathways (A). Influenza can pre-dispose to other infections by causing apoptosis of airway epithelial cells. Opportunistic bacteria then gain entry to the body’s interior (barrier breakdown, B). Influenza, and the inflammatory environment it induces, causes the up-regulation of bacterial adhesins making the lung stickier for other organisms (C). In addition to the lysis of respiratory epithelial cells, influenza is reported to induce apoptosis of neutrophils and macrophages that are crucial for bacterial clearance (immune subversion, D). Finally, influenza induces a long term state of responsiveness to subsequent TLR signalling and so bacteria go unrecognised for longer (TLR de-sensitisation, E).

that causes epithelial barrier disruption. An increase in bacterial adhesion molecules and apoptosis of anti-bacterial immune cells such as alveolar macrophages and neutrophils [126,132,133] is also apparent. Furthermore, IFNγ production during influenza infection decreases the phagocytic and anti-bacterial, capacity of alveolar macrophages [134]. The influenza encoded neuraminidase also contributes significantly to a localised immune suppression within the lung microenvironment, by cleaving sialic acid residues from IgA and γδ T cells; in the case of the latter, this may alter their potential to home to mucosal tissues [135]. However, considering the position of our innate immune rheostat in resolution it is likely that a de-sensitisation or blunting of alveolar macrophage responses to bacterial danger signals (Toll-like receptor ligands) [86] and an increase in CD200R [79] also contribute to this susceptible state. Influenza therefore raises the threshold for myeloid cell recognition of bacteria. We are not implying that the innate immune rheostat during resolution occurs in everyone infected with influenza. Some succumb to influenza infection, others do not. In a proportion of those that succumb a secondary infection with a bacterium may occur or not. Even in that event, some will handle the combined infection. However, a significant proportion will not and it is in those an adjustment of the innate immune rheostat may be beneficial. To support this, as we alluded to earlier, lung IL-10 production is enhanced and sustained after a secondary pneumococcal or meningococcal challenge in mice that have experienced an influenza infection, which in turn impairs the neutrophils ability to clear bacteria [136,137].

9. Concluding remarks

In inflammatory disease we often refer to a return to homeostasis. This implies that regulatory and inflammatory signalling pathways, Toll-like receptor expression, negative regulatory receptors, and even connective and neuronal support networks return to an original starting point. Mouse simulations of respiratory disease and clinical and epidemiological data clearly suggest this is not the case. This is not some form of “innate memory” as we do not believe individual innate cells are adjusted in isolation. Rather it is more likely that a combination of subtle alterations (in multiple pathways) leads to an alteration in the particular cells analysed. For example, alveolar macrophages have higher levels of CD200R long after resolution of inflammation because other cells (most likely epithelial cells) secrete higher levels of IL-10 and possibly TGFβ. The higher secretion of these cytokines is in turn likely to be a result of altered epithelial cell homeostasis that is dictated by other stromal elements or even altered innervation in the resolved lung. These modifications over time do make evolutionary sense as in some instances it provides a beneficial environment equipped to handle the next infection more professionally. In a proportion, however, a particular sequence of events, or the same events on a different genetic background, in different environmental conditions, may lead to a worse outcome including the development of fatal secondary bacterial pneumonia described above. The status of the innate immune rheostat in the lung may also explain the difficulty in generating efficacy to aerosolised vaccines. The
lung and gut possess a variety of overlapping strategies to prevent inflammation where possible. This anti-inflammatory environment will alter with time and those alterations may raise the threshold required for innate activation. We are only beginning to understand regulatory networks in the lung environment. It is clear the epithelium has a much bigger role to play than providing a (semi)-impermeable barrier. It is also clear that maintenance of homeostasis, even the altered homeostasis in inflammatory resolution, is an adaptable, active process. There are extensive gaps in our knowledge on the signalling events downstream of many receptors on alveolar macrophages and particularly the interplay between immune potentiators and regulators. A thorough understanding of these concepts will help explain the variability of infectious disease in man, provide possibilities for therapeutic intervention during inflammatory lung disease and may assist in the design of vaccine strategies that can bypass stringent immune regulation in mucosal tissues.

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