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ROLE OF CYTOKINES IN HYPEROXIA MEDIATED INFLAMMATION IN THE DEVELOPING LUNG

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1. ABSTRACT

The development of Chronic Lung Disease of Prematurity (CLD) has been associated with the use of hyperoxic conditions during ventilation. Inflammation has been demonstrated to contribute to the development of this disease, both on histological examination of diseased lungs, and by the use of bronchoalveolar lavage. Hyperoxia is believed to contribute to this inflammatory process by causing direct injury to epithelial and endothelial cells. The formation of reactive oxygen species is thought to result in production of cytokines. These act within a complex network, orchestrating an inflammatory response. Evidence for a role of cytokines in CLD has been inferred by studies in human infants showing increased concentrations of cytokines, growth factors and inflammatory cells at early stages in infants destined to develop CLD. These findings have been supported by the use of animal models of hyperoxic lung injury. The treatment of CLD is currently centered on the suppression of cytokine production. As understanding of this disease increases, more specific targets are being developed which aim to reduce the oxidative load on the lung, and prevent recruitment of inflammatory cells that are responsible for the tissue damage underlying this disease.

2. INTRODUCTION

Chronic Lung Disease of Prematurity (CLD) affects almost half of all infants ventilated below 32 weeks gestation and contributes significantly to the long term morbidity of preterm infants (1). It is variously defined and newer definitions are reported regularly reflecting the poor understanding of the risk factors and their importance in the pathogenesis of this disease. The commonest terms used are CLD, bronchopulmonary dysplasia (BPD) and more recently “new” BPD and “new” CLD have been used. Despite the various definitions, there are some features which are central to all definitions: CLD remains essentially a disease of preterm infants with the most extremely low birth infants at most risk of developing this disease. Secondly, the disease itself is defined by the dependency of the infant on oxygen. In most developed countries CLD remains rare in the more mature term or near-term infant. In this article, we have used the term CLD but appreciate that various terms including BPD are often used to describe the disease process.

Despite improvements in neonatal intensive care including the widespread use of exogenous surfactant and antenatal steroids over the last 10 years, there has been

little alteration in the incidence of this disease. In part, this is believed to be due to improvements in survival of extremely preterm infants who now proceed to develop CLD. It has also been noted that the nature of CLD has altered. Previously, CLD affected larger infants with severe Respiratory Distress Syndrome (RDS) who were exposed to high ventilatory pressures and oxygen concentrations, and was characterized by an inflammatory response to this treatment. Now, CLD affects extremely preterm infants who may not have significant RDS at birth but have immature pulmonary development. Exposure to the extra-uterine environment and to treatment modalities results in abnormal lung development (2). It is likely that there exists a spectrum between these two pathologies and that common underlying processes are occurring. Pathologies other than inflammation may contribute to this disease, emphasizing the multi-factorial pathogenesis of CLD. Disordered vascular development and dysregulated alveolization are examples of alternate etiologies although inflammation may contribute to the underlying disease process. We will review the evidence for the inflammatory processes in CLD, together with current theories regarding the mechanisms that underlie this disease, concentrating on oxygen toxicity in the preterm infant.

3. THE ROLE OF CYTOKINES IN ACUTE LUNG INJURY

3.1. Initiation of the inflammatory response

Inflammation plays a role in host defense. It has evolved to protect against a multitude of insults particularly microbial pathogens and abnormal host cells. However it has become apparent that the inflammatory response can be debilitating whilst being mobilized excessively against self-limiting insults, or chronically against continued stimuli such as ventilation, and thus prove counterproductive.

The response of any tissue to injury can be classified according to the stages of an inflammatory response. After initial recognition of injury, a coordinated response must be initiated both locally and remotely, to allow the recruitment of effector cells. These have a role of containing the injury. The predominant effector cell during early stages of inflammation is the polymorphonuclear neutrophil, which once recruited and activated, either phagocytoses bacteria, or releases granule contents into the local environment. Subsequent cellular influx include macrophages which at times play an initial role in coordinating effector response, but with time also have a role in controlling and finally down regulating inflammation. Once removal of the offending agent has been accomplished, a reparative phase follows, re-establishing normal structure and tissue function, or alternatively, resulting in fibrous scar tissue.

Initial injury can be due to a number of agents including hyperoxia, or infection. The classical response to endotoxin injury commences with the recognition of lipopolysaccharide (LPS). This is a component of the bacterial cell wall, and specifically binds CD14 sites located on macrophages. These cells act as the initiators of the inflammatory response and when appropriately stimulated, respond with the production of signaling molecules termed cytokines, which share the ability to alter the functional characteristics of target cells. Cytokines are a diverse collection of peptides, which interact as part of a complex network to initiate and coordinate the inflammatory and reparative processes.

Cytokines have been categorized into various hierarchical categories. Traditionally these have been based upon the source of the cytokines, hence interleukins (IL) are derived from leukocytes, lymphokines from lymphocytes, and monokines from monocytes. These categories have become more arbitrary as investigations have progressively revealed that all cells are capable of producing a range of cytokines. It is this that enables differentiation from hormones which are produced by specialized cells and tissue, and act upon specific end-organs. The early response cytokines tumor necrosis factor-alpha (TNF-alpha) and IL-1 (3) are produced by the response cells including macrophages, endothelial cells, epithelial cells, fibroblasts and smooth muscle cells within the lung. IL-1 is a 17kDa protein produced predominantly by monocytes and is induced by other cytokines, particularly TNF-alpha. TNF-alpha is a 17kDA protein, which derived its name from the observation that the injection of this protein into tumor tissue leads to rapid destruction of small supply blood vessels within the tumor leading to involution. These together are classed as proinflammatory cytokines and act on macrophages to induce their own production by positive feedback. Their ability to recruit other macrophages locally enables a rapid amplification step, priming the region with numerous, activated macrophages.

3.2. Chemoattractants

Recruitment of neutrophils is mediated predominantly via the CXC chemokines (*chemo*tactic cyto*kines*) (4). These polypeptides range in size from 7-10kDa and are structurally related by the presence of four cysteine residues. Grouping into families is determined by differences in the cysteine arrangement. CC chemokines, which possess adjacent cysteine residues, include Monocyte chemotactic protein-1 (MCP-1), Macrophage inflammatory protein (MIP) and RANTES (Regulated upon Activation, Normal T cell Expressed and Secreted). These are chemoattractant for monocytes, lymphocytes, mast cells and eosinophils.

The CXC family possesses an amino acid residue between the first two cysteines. They are further subdivided into ELR (+) or ELR (-) groups depending on the presence of the Glu-Leu-Arg motif. ELR (+) chemokines are attractant for neutrophils and act as angiogenic factors (5). They include Growth Related Oncogene-alpha (GRO-alpha)? but Interleukin-8 (IL-8) is the most potent chemokine for neutrophils. ELR (-) chemokines include Platelet Factor 4 and are attractant to monocytes and inhibit angiogenesis.

The migration of neutrophils to the area of injury is mediated by a series of processes. The adherence of neutrophils to the endothelial wall by attachment of the

neutrophil ligand L-selectin, to P- and E-selectins expressed on endothelial surfaces, produces slowed movement of neutrophil's at these vessel sites, termed 'rolling'. The expression of L-selectin by endothelial cells is upregulated at inflamed areas due to the effect of TNF-alpha and IL-1. Subsequent firm adherence of neutrophil's and migration across the endothelium is partly mediated by the beta2-integrins, e.g. CD11b/CD18, which are upregulated on the neutrophil surface after activation, usually in response to IL-8. The beta2-integrins attach firmly to the receptor, ICAM-1 (intercellular adhesion molecule) expressed on endothelium (6). In addition, the lipid mediators Platelet Activating Factor (PAF) and Leukotriene B4 (LTB4), together with complement, all of which are produced as part of an inflammatory response, act to increase vascular permeability.

The expression of these facilitators of neutrophil migration are increased by the action of interleukin-6. This 16-21kDa glycoprotein has many sources including macrophages, endothelial cells, smooth muscle cells (SMC's) and fibroblasts, in response to proinflammatory cytokines. Although IL-6 acts systemically to produce an acute-phase response, it also has been shown to have a negative feedback response, resulting in decreased production of TNF-alpha and IL-1 in vitro.

3.3. Regulation of the inflammatory cascade

The proinflammatory cascade is regulated to prevent an uncontrolled inflammatory response. In part, this is provided by the presence of a group of anti-inflammatory mediators that provide a counterbalance to the proinflammatory agents. These include IL-4, IL-10, IL-13, Transforming Growth Factor-beta (TGF-beta), soluble TNF-alpha receptor antagonist (sTNFra), Interleukin 1 receptor antagonist (IL-1ra) and IL-8ra.

Preterm neonates are able to mount a significant inflammatory response, yet are also predisposed to infection. Regulation of the inflammatory response in neonates has shown that the T-cell response is biased towards Th-2 cells, responsible for the production of IL-4, IL-5, IL-10, IL-13, TNF-alpha and GM-CSF. In contrast, Th-1 function is suppressed, reducing Interferon responses (7,8).

IL-10 and IL-13 are produced in response to inflammation, and provide a negative feedback to suppress production of pro-inflammatory cytokines. The main sources of IL-10 are B- and T- lymphocytes. The soluble receptor antagonists, sTNF – RI and – RII, IL-1ra and IL-8ra, bind their specific agonists without activating the cell signaling pathways which are normally activated when the ligands bind to their membrane bound receptors.

Activated macrophages secrete TGF-beta, a growth factor that is a major effector of tissue repair. TGF-beta?acts as either a pro- or anti-inflammatory agent (9), depending on its local concentrations. Additionally it inhibits protease synthesis whilst promoting anti-protease production, thus reducing the degradation of extracellular matrix, and upregulates the activity of fibroblasts, inducing the laying down of collagen and the formation of new matrix and/or scar tissue. It is postulated that an excess of TGF-beta, may result in excess fibrosis (10). A deficient ability to mount a TGF-beta response, can however, result in an enhanced inflammatory response, thus, there appears to be a fine balance for optimal repair (11). Together with the anti-inflammatory effects of cortisol, produced as part of the systemic stress response, it is the eventual increase in these mediators together with a waning in the proinflammatory cytokines that leads to the resolution phase of inflammation.

4. EFFECT OF OXYGEN ON THE DEVELOPING LUNG

Oxygen has long been recognized as being toxic to the lung. The extents of its effects are determined by its concentration, and the susceptibility of the host. The latter is greatly influenced by the stage of development of the subject. In adults, the administration of 100% oxygen to the lung for prolonged periods leads to the development of Acute Respiratory Distress Syndrome (ARDS). An initial destruction of endothelial cells is followed by epithelial type 1 cell necrosis. This results in an alveolar leak, resulting in diffuse exudation of plasma into alveolar spaces. Accompanying this cell injury is the release of pro-inflammatory cytokines and subsequent recruitment into the lung of inflammatory cells. Release of cytokines such as IL-6 results in a systemic acute phase response. These pathophysiological changes result in the clinical correlate of ARDS, characterized by severe respiratory failure with diffuse pulmonary infiltrates in the absence of congestive heart failure (12).

It has long been recognized that neonates of most species are more resistant to the toxic effect of hyperoxia than adults. Classically in infant CLD, the triad of prematurity, hyperoxia and ventilator-induced lung injury have been regarded as prerequisite risk-factors. This observation has been demonstrated in animal models with the increased survival of newborn rats exposed to 100% oxygen when compared to adult counterparts who all perish within 1 week of exposure (13).

More recent studies have demonstrated the link between hyperoxia and inflammation in the developing animal lung. Warner *et al* (14), utilized the newborn mouse as a model of hyperoxic lung injury as alveolar development progresses in the early postnatal period. Exposure of these animals to 85% oxygen for 1 month resulted in 40% mortality. Survivors demonstrated decreased alveolar septation with increased terminal airspaces and evidence of lung fibrosis- findings similar to those found in CLD. Lung lavage studies have shown that hyperoxia results in a neutrophilic infiltrate, peaking 2 weeks after exposure to hyperoxia. Analysis of neutrophil mRNA revealed an upregulation in expression of proinflammatory cytokines IL-1beta and Macrophage Inhibitory Protein (MIP-1alpha).

5. EVIDENCE FOR A ROLE OF INFLAMMATION IN CLD

5.1. Histological evidence of inflammation in CLD

Evidence has accumulated from a number of studies linking inflammation to the development of CLD. These initially relied on interpretation of histological samples of lung obtained from infants dying from CLD. These, however, represented the most severely affected babies. They do nevertheless demonstrate inflammatory cellular infiltrates in the lungs of ventilated babies when compared to those who succumbed to non-respiratory causes. (15) These infants were characteristically preterm babies born at gestations of 28-34 weeks who had been exposed to high-pressure ventilation and to high concentrations of oxygen. Histology confirmed the presence of interstitial fibrosis, alveolar wall thickening and smooth muscle hypertrophy. Recent studies looking at infants who were delivered at more immature gestations (23-28 weeks) have revealed different features. A simplification of the alveolar unit predominates with less striking inflammatory changes. In particular, there appears to be a failure to develop secondary alveolar septation and an associated pruning of the microvasculature. It is hypothesized that this failure of vascular development leads to this malseptation (16). These findings are less likely to be due to hyperoxic lung damage, and more likely to be related to both ante- and postnatal inflammation resulting in dysregulated lung development.

5.2. Analysis of bronchoalveolar lavage from infants with CLD

5.2.1. Cellular infiltrates

Techniques to look at evidence for inflammation in living human preterm infants have relied principally on bronchoalveolar lavage (BAL). This methodology involves the instillation of saline via a catheter wedged into a lobar bronchus followed by the aspiration of terminal airway and alveolar contents (17, 18). This contrasts with the technique of tracheal aspiration, which predominantly samples the larger proximal airways. As with histological samples, an inflammatory infiltrate has been repeatedly demonstrated in BAL from preterm infants with lung disease. It has consistently been shown that persistence of neutrophils in the lung beyond 7-10 days of age is associated with the development of CLD (19-21). Although there were no differences in lymphocyte numbers noted in the study by Murch *et al*, activated macrophages denoted by expression of the RM/3-1 marker, were similarly found to be increased in the BAL of babies with CLD, peaking at one week of age and subsequently reducing in numbers over the following weeks (20). This contrasted with neutrophil numbers in the same lavage samples, whereby progressive CLD was marked by an excess number of neutrophil's over macrophages. This supports earlier work demonstrating an increased presence of macrophages at 96 hours in babies with uncomplicated RDS as well as those with developing CLD, but with a relative paucity in macrophages in CLD patients at 4 weeks of age (22).

The activity of recruited neutrophils has been estimated by study of their released local effectors, principally elastase. The proteolytic properties of this enzyme are likely to impair the septation process in the lung necessary for normal alveolar development. The activity of elastase is counterbalanced by the presence of alpha1-protease inhibitor (alpha1-PI). At birth, the ratio of elastase and alpha1-PI in the BAL of normal controls and infants with RDS are similar. In infants who progress to develop CLD, however, a raised elastase/alpha1-PI ratio develops within the first 48 hours of life and is maintained for several weeks (22). This reflects increased elastase production by neutrophil's, reduced macrophage contribution to the alpha1-PI pool, and inactivation of alpha1-PI by the oxidizing effect of toxic oxygen radicals (23). Matrix Metalloproteinases (MMP's) are a specialized subgroup of proteinases that form an important link in controlling tissue degradation during lung injury. MMP-12 deficient mice develop a less intense inflammatory response and tissue damage in response to cytokine stimulation than normal counterparts, illustrating the potential damage from MMP overproduction (24).

5.2.2. Chemoattractants

The process for leukocyte recruitment has also been investigated. Chemotaxis for monocytes and macrophages are in part mediated by beta-chemokines MIP-1alpha and RANTES. The former has been demonstrated to be raised in BAL from patients who go on to develop CLD (20). Chemoattractants for neutrophils include C5a (Complement-5a), Granulocyte-Colony Stimulating Factor (G-CSF), Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), LTB4 (Leukotriene B4), IL-16 and IL-8, the latter being the most potent. C5a is a product of complement activation and is correlated with increased neutrophil activity. G-CSF and GM-CSF are produced by macrophages and promote clonal expansion of airway neutrophils and macrophages (25). LTB4 is itself a by-product of activated neutrophils and acts to recruit more neutrophils in a positive feedback loop. IL-16 is a potentially important CD4+ cell chemoattractant, resulting in recruitment of macrophages, and subsequently, neutrophils. The aforementioned neutrophilic chemoattractants, have been found to be increased in epithelial fluid of patients developing CLD (25-28) .

IL-8 is a neutrophil attractant and has been consistently demonstrated to be raised in association with CLD when compared to infants who recover from RDS. We noted a significant rise in IL-8 BAL concentration at 10 days in CLD (21), findings supported by Groneck et al (29) and Baier (30). Munshi (31) noted a significant rise in IL-8 in babies with RDS progressing to CLD as early as 1-3 days postnatally. Differences maybe partly explained by the utilization of tracheal aspirates by Munshi rather than bronchoalveolar lavage, resulting in sampling from central airways rather than distal lung (32). The accumulation of IL-8 appears predominantly confined to the lungs. Serum concentrations of IL-8 do not appear to differ between CLD and RDS subjects and are an order of magnitude below lavage concentrations (21).

The role of neutrophils in CLD is further supported by evidence of increased production of adhesion molecules.

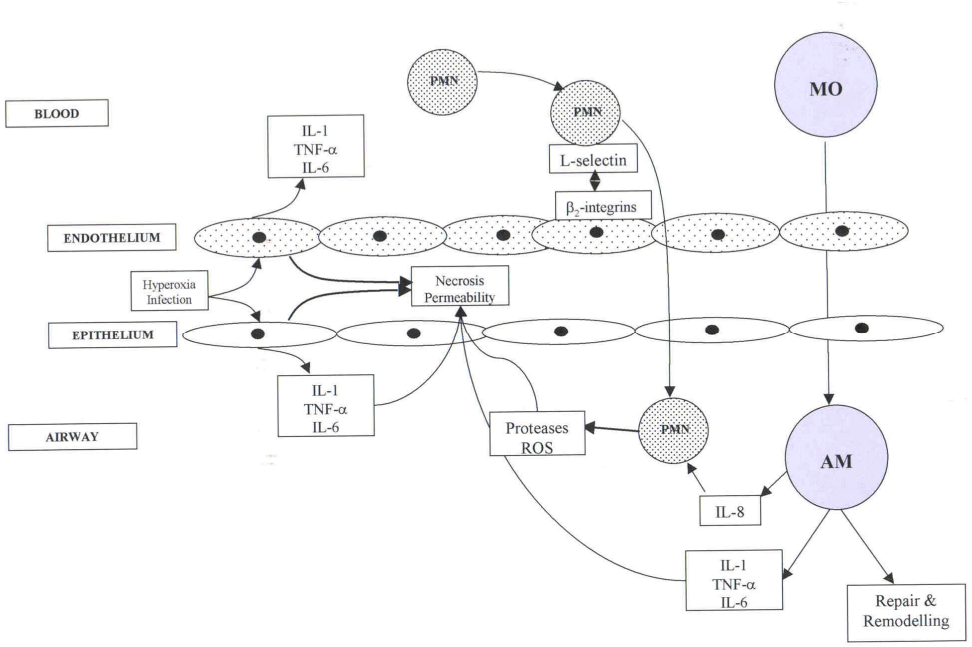

Figure 1. The figure shows a simplified version of the events that are likely occur in infants at risk of developing CLD. Risk factors such as oxygen therapy, ventilator-induced injury and infection are likely to lead to the recruitment of neutrophils (PMN) to the lungs where the production of proteases and reactive oxygen species (ROS) results in lung tissue damage. The cells of resolution and repair namely alveolar macrophages (AM) are derived from peripheral blood mononuclear cells (MO) are potently producers of cytokines both pro-inflammatory, chemoattractants and growth factors which are important in repairing the acute lung injury. Many of the growth factors, which are important in normal repair and remodeling, are also important in normal lung growth. Abbreviations: IL-1 – interleukin-1beta, IL-6 – interleukin-6, IL-8 – interleukin-8.

Analysis of BAL from infants with CLD reveals a persistent increase in soluble L-selectin, a marker of neutrophil recruitment via the 'rolling' mechanism (33).ICAM-1 and its ligand CD11b/CD18, which is expressed by neutrophils, are important for extravasation of leukocytes in to the lung. The soluble form of ICAM (sICAM) is increased in BAL from infants with CLD compared to those who develop and recover from RDS (21). Similarly, neutrophil's from these infants express greater amounts of CD11b/CD18 than neutrophil's from infants whose lung disease resolves. This increase in sICAM has also been found in serum of babies with CLD at 10 days of age (34).

5.2.3. Proinflammatory cytokines

Initiation of the inflammatory response is mediated by the proinflammatory cytokines TNF-alpha?IL-1 and IL-6. TNF-alpha, which possesses the ability of promoting the expression of IL-1beta and IL-6, can be produced in response to infective agents via LPS, or injurious agents such as ventilator-induced lung injury or hyperoxia. Antenatal inflammatory response is believed to be related to infection in the amniotic fluid by organisms such as *Ureaplasma urealyticum*, which have been linked to the development of CLD (35-36) , in particular the 'new' form of CLD, which can occur in the absence of hyperoxic insult. Raised concentrations of TNF-alpha in BAL from patients who developed CLD has been found at 14 days of age, but has also been described as early as 3 days together with increased presence of immunoreactive cells in BAL (37-39). IL-1 has been shown to be predictive of CLD in the first week of life (38,40-2). (Figure 1)

IL-6, which has both pro- and anti-inflammatory properties has been reported by several groups to be associated with CLD when detected in high concentrations in the first week of life (31,37,40,43). However, changes in either IL-1 or IL-6 were not detected by a similar study by Kazzi (44).

5.2.4. Regulators of inflammation

Evidence has been sought for a failure in normal anti-inflammatory and resolution processes as a contributor to the development of CLD. Initial speculation that inability to produce IL-10 in preterm infants leads to an increased risk of CLD was put forward by Kwong (45) and Jones (46). Bronchoalveolar lavage of preterm infants revealed undetectable IL-10. Cells extracted during this process were shown not to express IL-10. Conflicting data showing that extreme prematurity was associated with increased IL-10 in BAL compared to more mature counterparts was demonstrated by Jonsson (47). Indeed, in this study, BAL IL-10 correlated with the development of CLD (48). This finding, associating IL-10 with CLD, was supported by McColm (49), who demonstrated BAL IL-10 protein in a group of babies less than 29 weeks gestation. Further investigations into the prognostic significance of IL-10 in CLD are required.

TGF-beta has more conclusively been associated with the development of CLD. Studies have demonstrated an increased presence of TGF-beta protein in lavage from the lungs of infants who develop CLD, within the first week of life (50). These remain elevated in chronically ventilated babies with a need for home oxygen (51). Physiological release of TGF-beta is intended to be short-lived, however, the maintenance of an inflammatory stimulus e.g. hyperoxia, infection, leads to an exaggerated response that is perpetuated by the ability of TGF-beta to stimulate its own secretion (52).

6. THE MODULATORY EFFECT OF OXYGEN ON CYTOKINES AND GROWTH FACTORS

The association between hyperoxia and CLD is believed to stem from oxidant stress. The increased availability of oxygen results in leaking of reactive oxygen species (ROS's) from the mitochondrial electron chain. This oxygen load cannot be dealt with by the immature antioxidant enzyme system present in preterm infants when compared to their term counterparts (53). The formation of these oxygen radicals also results from the process of hypoxia/reoxygenation, whereby the conversion of xanthine dehydrogenase to oxidase results in the generation of superoxide anions (54). Another source of ROS, once inflammation has been initiated, is from activated neutrophils recruited to the lungs in RDS. Via the NAPDH oxidase pathway, these cells produce and release of hydrogen peroxide, superoxide and hydroxyl radicals.

A number of pathways exist by which reactive oxygen species exert their effect. A major determinant is the direct toxicity they possess, inherent from their ability to react with cellular constituents. In particular, lipid peroxidation leads to cellular membrane disruption, followed by cellular necrosis. The release of a variety of intracellular constituents have chemotactic properties, resulting in the recruitment of neutrophil's to the lung (55). Evidence for increased lipid peroxidation in early CLD infants has been demonstrated by Pitkanen (56), by the measurement of the expired lipid by-products, ethane and pentane.

ROS have been shown to assist in recruiting inflammatory cells by inducing the prolonged expression of specific neutrophil binding proteins on endothelial cell surfaces in particular Endothelial Leukocyte Adhesion Molecule-1 (ELAM-1) (57) and Granule Membrane Protein-140 (GMP-140) (58). This upregulation of specific protein expression provides an example of the role ROS have in signal transduction (59), in particular the activation of nuclear factor-kappa-B (NFkappaB) (60). This transcription regulatory protein is located within cytoplasm, bound to an inhibitory protein. This is cleaved to its active form by ROS, resulting in attachment of the activated NFkappaB to its promoter sites on specific genes in the cell nucleus, enabling their transcription. Many immunomodulatory targets exist for NFkappaB including transcription of TNF-alpha? IL-1, IL-6, IL-8, MIP-1alpha and the cell adhesion molecules. A secondary effect of NFkappaB activation is the inhibition of neutrophil apoptosis via transduction of cell death regulators. Apoptosis is considered an important contributor to neutrophil removal (61), thus inhibition of this process prolongs inflammation.

The mechanisms by which oxygen has a regulatory effect on cytokine expression may give insights to potential future therapies specifically targeted to these mediators of inflammation.

7. THERAPEUTIC MODULATION OF HYPEROXIA-INDUCED INFLAMMATION IN CLD

The role of cytokines in coordinating inflammation have made them a target for potential therapies for CLD. Drug therapies has focused on removing stimuli for cytokine expression, suppression of production, or blocking their terminal effect. In addition, the use of cytokines themselves has become a therapeutic option.

7.1. Infection

The use of antenatal antibiotics to prevent the inflammatory cascade associated with subclinical infection during preterm labor was believed to be a suitable and simple means for preventing CLD. This hypothesis was recently tested by the ORACLE trial (62). The use of antibiotics in preterm labor was not shown to significantly reduce the incidence of CLD, although the use of erythromycin when rupture of membranes had occurred led to a small reduction in CLD on subgroup analysis (63,64). An increased incidence of necrotising enterocolitis associated with the use of Augmentin during this trial reminds us of the potential hazards of any therapy.

The role of postnatal antibiotics has also been investigated. *Ureaplasma urealyticum* has been associated both ante- and postnatally with the development of CLD. Treatment with erythromycin in small studies has not altered the development of this disease, nor has it altered cytokine profiles in BAL from treated infants (65).

7.2. Antioxidants

Supplementing the immature antioxidant system of the preterm infant has been attempted using a number of pharmacological agents including Vitamin E, Vitamin A and superoxide dismutase (SOD) (66,67). Vitamin A supplementation has been associated with a modest reduction in CLD amongst survivors at 36 weeks, however administration is via repeated intramuscular injections (68). No clinical improvement has been demonstrated after the use of Vitamin E in infants less than 1500g (66).

Animal studies have shown a reduction in lung injury after intratracheal administration of antioxidants including SOD, alpha ?ocopherol, and N-acetylcysteine (69,70). Human studies have demonstrated a reduction in inflammatory mediators with the use SOD, but as yet no improved clinical outcomes have been demonstrated conclusively other than reduced admissions for respiratory illnesses in the first year of life (71-73) . This limited effect may be secondary to limited ability to deliver these agents to the lung. Newer methods of delivery may show promise,

in particular, recombinant human CuZn SOD used in combination with a surfactant vector (74,75).

7.3. Corticosteroids

These currently provide the mainstay of pharmacological treatment of CLD-associated inflammation. Steroids interact via glucocorticoid receptors and subsequent regulation of several genes, which code for proteins with anti-inflammatory actions. This may be upregulation of production e.g. lipocortin-1 synthesis, resulting in inhibition of prostaglandin and PAF production. More commonly, inhibition of the expression of pro-inflammatory genes occurs e.g. IL-1, IL-6, IL-8, TNF-alpha, ?RANTES, MIP-1alpha ?and adhesion molecules (76).

The use of antenatal steroids has been proven an effective therapy for preterm infants (77). Although outcomes for the incidence of CLD have not been altered by its use, it is likely that this may be related to increased survival of the more immature infants who are more likely to develop CLD. There is no evidence for a direct antenatal anti-inflammatory effect of antenatal steroids. However, an induction of antioxidant enzymes and surfactant proteins are known to be promoted by antenatal corticosteroids. Concerns have been raised more recently that the overuse of antenatal steroids, particularly repeated courses, are associated with worse respiratory outcomes (78).

The use of systemic steroids postnatally in preterm infants has been associated with reduction in inflammatory markers and neutrophils in BAL, and has been shown to result in hastened extubation (79). Optimal timing and dosage of steroid administration remains controversial with a failure to demonstrate increased survival in infants given postnatal steroids and from the association of steroid use with later neuromotor disability (80-82). Concerns have also arisen regarding the detrimental effect steroids have on normal postnatal alveolar development (83). Animal studies have demonstrated that steroids during the postnatal period impair the normal septation process, reducing overall alveolar numbers (84). Non-respiratory adverse effects of systemic steroid administration include immunosuppression, cardiac hypertrophy, hyperglycemia, growth failure and hypertension. The concerns related to these systemic side effects, have led to attempts to utilize the inhaled route of administration. In one study, the use of inhaled steroids in preterm infants was found to reduce concentrations of inflammatory cytokines in BAL fluid and reduce need for systemic steroids subsequently (85). A reduction in the incidence of CLD has not been demonstrated in larger studies addressing this question, and at present, their use is not widespread (86,87).

7.4. Non-steroidal anti-inflammatory drugs

A number of non-steroidal anti-inflammatory agents have been assessed for the prevention and treatment of CLD. Ibuprofen has theoretical potential to block inflammatory prostaglandin pathways. However, *in vitro* studies utilizing infant monocytes incubated with ibuprofen, show an increase in pro-inflammatory cytokine production and decrease in anti-inflammatory cytokines (88). A clinical study comparing the use of ibuprofen with placebo in preterm infants showed only a trend towards reduced ventilator days in the treatment group (89). It remains to be tested if anti-inflammatory effects of drugs such as ibuprofen and indomethacin will prevent the development of CLD.

Cromolyn acts to stabilize mast cells, and reduce neutrophil chemotaxis and activity (90, 91). An early study looking at the use of nebulised cromolyn from day 1 in babies under 1000g (92), in the pre-steroid/surfactant era, showed no difference in survival or incidence of CLD. A later study where cromolyn was administered from day 3 (93), again showed no reduction in the development of CLD. However, lung lavage samples taken during this study showed significant reduction in TNF-alpha?and IL-8 concentrations, implying an effective anti-inflammatory effect. This illustrates the difference between clinical and cellular outcome measures that can exist and reinforces the need for well-designed clinical studies with sufficient power to address specific underlying hypothesis.

7.5. Anti-inflammatory cytokines

The culture of tracheal cells from infants with RDS, demonstrates a dose dependent suppression of IL-1? TNF-alpha, ?L-6 and IL-8 protein production by recombinant human IL-10 (45,94). The administration of this cytokine to mice exposed to bacterial pneumonia, results in decreased lung injury and mortality (95). The upregulation or administration of IL-6 has become another potential target. Transgenic mice, over expressing IL-6 and IL-13 appear to be protected from hyperoxic lung injury and resultant death (96). This however is at the expense of remodeled airways with emphysematous lungs (97). At present, these treatment modalities remain experimental. A future role for the treatment of human disease remains to be seen.

7.6. Chemoreceptor antagonists

Antagonism of cytokines can be produced directly using antibodies either to the cytokine molecule, or to receptor blockers.

CXCR2 antagonists target the CXC chemokine receptors (type 2) present on neutrophil's. These have been administered to rats exposed to hyperoxia to block IL-8 chemoattractant effects. Together with reducing inflammatory infiltrates, there was prevention of alveolar septal wall thickening (98, 99). Similarly, in rats with immune-complex mediated lung disease, the administration of antibody to IL-8 reduces inflammatory responses in lung injury via blockage of E-selectin mediated recruitment (100). Using a hyperoxia-ventilation injury model in rabbits (101), the administration of IL-1 receptor antagonists reduced lung albumin and elastase concentrations together with neutrophil influx. There was however, no improvement in respiratory dynamics in this model.

8. SUMMARY

Chronic Lung Disease of Prematurity occurs commonly in ventilated preterm infants. Hyperoxia has

been determined to be a significant risk factor for the development of CLD. The mechanisms by which this disease process occurs has become progressively elucidated with time by the use of *in vitro* and vivo investigations. Preterm infants are particularly vulnerable to oxygen toxicity as a consequence of an immature antioxidant system. Complex cytokine networks play a crucial role in the pathophysiology of CLD, and provide a target for therapeutic modulation. A number of treatments have been investigated which down-regulate the immune response in a non-specific manner e.g. corticosteroids and non-steroidal anti-inflammatory drugs. These treatments have many unwanted side-effects limiting their usefulness. More specific anti-cytokine treatments are being developed such as chemokines receptor antagonists. It is hoped these novel experimental interventions will provide additional means to treat this disease process.

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