Factors driving the aspirin exacerbated respiratory disease phenotype

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ABSTRACT

Background: *Aspirin-exacerbated respiratory disease (AERD) is explained in part by overexpression of 5-lipoxygenase and leukotriene C_4 synthase (LTC_4S), resulting in constitutive overproduction of cysteinyl leukotrienes (CysLTs) and driving the surge in CysLT production that occurs with aspirin ingestion. Similarly, AERD is characterized by the overexpression of CysLT receptors. Increased levels of both interleukin (IL)-4 and interferon (IFN)-γ are present in the tissue of AERD subjects. Previous studies demonstrated that IL-4 is primarily responsible for the up-regulation of LTC_4S by mast cells.*

Methods: *Literature review.*

Results: *Our previous studies demonstrated that IFN-γ, but not IL-4, drives this process in eosinophils. These published studies also extend to both IL-4 and IFN-γ the ability to up-regulate CysLT receptors. Prostaglandin E_2 (PGE_2) acts to prevent CysLT secretion by inhibiting mast cell and eosinophil activation. PGE_2 concentrations are reduced in AERD, and our published studies confirm that this reflects diminished expression of cyclooxygenase (COX)-2. A process again that is driven by IL-4. Thus, IL-4 and IFN-γ together play an important pathogenic role in generating the phenotype of AERD. Finally, induction of LTC_4S and CysLT1 receptors by IL-4 reflects in part the IL-4-mediated activation of signal transducer and activator of transcription 6 (STAT6). Our previous studies demonstrated that aspirin blocks trafficking of STAT6 into the nucleus and thereby prevents IL-4-mediated induction of these transcripts, thereby suggesting a modality by which aspirin desensitization could provide therapeutic benefit for AERD patients.*

Conclusion: *This review will examine the evidence supporting this model.*

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Aspirin-exacerbated respiratory disease (AERD) or Samter's triad was originally defined by the presence of nasal polyps, aspirin sensitivity, and asthma.[1] It is now recognized that this disorder is characterized by hypersensitivity not only to aspirin but also to other nonselective cyclooxygenase (COX) inhibitors.[2–4] Asthma is not always present and thus the preferred terminology AERD. Other characteristics include the less common association with atopy,[3,5] hypereosinophilia, and a tendency to develop de novo in adulthood.[3,5–7] Aspirin hypersensitivity is found in as many as 10%–20% of adult asthmatics and up to 30% of asthmatics with nasal polyposis.[5,8] When asthma is present in this disorder, it often becomes severe and is associated with aggressive airway remodeling.[9] Similarly, the sinusitis present in this disorder is often severe and associated with complete or near complete sinus opacification.[7]

A central feature of AERD is its association with profound overproduction and overresponsiveness to cysteinyl leukotrienes (CysLTs)[10,11] occurring concomitantly with a profound underproduction and underresponsiveness to prostaglandins.[12–14] These CysLTs have important proinflammatory and profibrotic effects that contribute to the asthma severity and to the extensive hyperplastic sinusitis and nasal polyposis.[7,15,16] And, conversely, the down-regulation of prostaglandin pathways reduces the constraints that would normally act to attenuate these proinflammatory pathways.[17] This review will focus on the dysregulation of these respective pro- and antiinflammatory pathways and the cytokine mechanisms that underlie this dysregulation and, finally, will discuss implications of aspirin desensitization as a therapeutic intervention that acts by altering these pathways.

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CYSTEINYL LEUKOTRIENE OVERPRODUCTION AND OVERRESPONSIVENESS IN AERD

AERD is characterized by the constitutive overproduction of CysLTs and a massive, potentially life-threatening, further surge in CysLT production in response to aspirin and other nonselective COX inhibitors that block COX-1.[18] This includes not only nonselective nonsteroidal antiinflammatory drugs (NSAIDs) but also other inhibitors of COX-1, including alcoholic products.[19,20] Overproduction of CysLTs in AERD reflects the increased expression of its primary synthesis enzymes 5-lipoxygenase and especially leukotriene C_4 synthase (LTC_4S). Up-regulation of these enzymes is observed in the lungs, sinuses, and nasal polyps of AERD subjects, localized in large part to the infiltrating eosinophils, and resident mast cells.[12,15,21,22]

AERD subjects also demonstrate an increased sensitivity to CysLTs,[23] reflecting in part their up-regulation of CysLT 1 receptors.[24] The two originally characterized CysLT receptors were distinguished by their differing potency for the CysLTs: CysLT1 receptors primarily respond to LTD_4, whereas CysLT2 receptors respond equally to LTD_4 and LTC_4. However, this pattern could not explain a body of literature demonstrating the capacity of LTE_4, and not either of these other CysLT receptors, to drive smooth muscle contraction and proinflammatory influences on both airway explants[25] and, via inhalation challenges, on the airway itself of AERD subjects.[23,26,27] The relative insensitivity of either CysLT1 or CysLT2 receptors to LTE_4, in contrast to the sensitivity of AERD subjects to this lipid mediator, led to the exploration for and ultimate identification of additional CysLT receptors that selectively respond to LTE_4.[28–30] CysLT type 1 receptors are prominently expressed on airway smooth muscle,[31] and these receptors do mediate much of the CysLT-induced bronchospasm associated with aspirin challenges or desensitizations,[32–35] as evinced by the ability of leukotriene receptor antagonists to attenuate much of the bronchospasm that occurs with these procedures. Thus, AERD is characterized by enhanced sensitivity to leukotrienes, reflecting in part the overexpression of CysLT1 receptors. The enhanced responsiveness of these subjects to LTE_4 is intriguing, although as of now, the expression and function of putative LTE_4 receptors in this disorder remains unstudied.

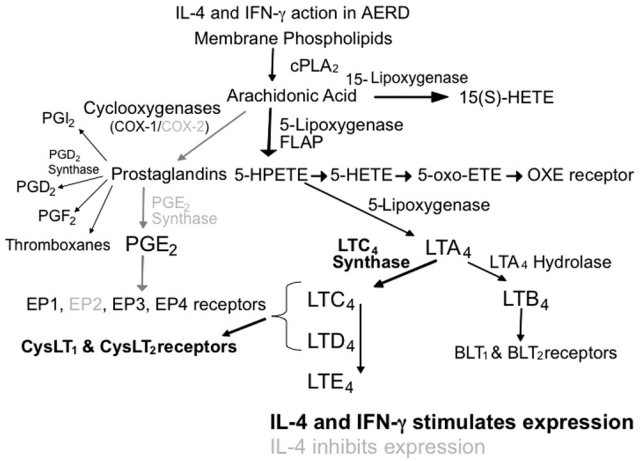

Figure 1. Summary of IL-4/IFN-γ activity on the leukotriene and prostaglandin synthesis pathways. Activation of gene synthesis by IL-4/IFN-γ is shown in boldface while inhibition of gene synthesis by IL-4 is shown in grey.

PROSTAGLANDIN E_2 (PGE_2) AND PGE_2 RECEPTOR DYSREGULATION IN AERD

PGE_2 displays both pro- and antiinflammatory functions reflecting its ability to interact with four distinct receptors (EP1–EP4), each having various activating or inhibitory functions. However, it is the role of PGE_2 acting through antiinflammatory EP2 receptors to block eosinophil and mast cell degranulation that is central to the pathogenesis of AERD. AERD patients constitutively display low levels of PGE_2[12,36] attenuating the antiinflammatory constraints provided by this lipid. The further reduction of tissue PGE_2 concentrations by aspirin and other NSAIDs through COX-1 inhibition precipitates the activation of eosinophils and mast cells in AERD, as demonstrated by the ability of an infusion of PGE_2 to protect against these reactions.[37,38] This sensitivity of AERD patients to low tissue PGE_2 concentrations is amplified by their reduced expression of the antiinflammatory EP2 receptor.[14]

Several studies have investigated the mechanism behind the reduced levels of PGE_2 in AERD and, perhaps not surprisingly, have correlated this with a decrease in the responsible upstream metabolic enzymes. The production of PGE_2 from arachidonic acid involves the sequential synthesis of PGG_2/PGH_2 by the two COX enzymes (COX-1 and COX-2) followed by the synthesis of PGE_2 by the microsomal PGE_2 synthases (mPGES-1 and mPGES-2) and cytosolic PGE_2 synthase. It is mPGES-1 that is most relevant to PGE_2 production in inflammatory disorders such as AERD, because it is the enzyme primarily functionally coupled to COX-2.[39] COX-2 mRNA and protein expression are markedly diminished in AERD.[12,13,40] Our studies have confirmed this diminished expression of COX-2.[41] We found no significant change in COX-1 and a trend toward diminished mPGES-1 expression.

Diminished COX-2 expression and the reduced capacity to synthesize PGE_2 contributes to the severity of inflammation observed in AERD and accentuates the sensitivity of these individuals to the inhibition of PGE_2 synthesis associated with aspirin and other NSAIDs. With this relative absence of COX-2, AERD subjects become dependent upon COX-1 for the PGE_2 that is necessary to restrain mast cell and eosinophil activation. Most AERD patients tolerate selective COX-2 inhibitors, supporting this concept regarding the unique importance of COX-1-derived PGE_2. In summary, AERD represents a state characterized by constitutive overexpression and overresponsiveness to CysLTs occurring concomitantly with diminished expression and underresponsiveness to PGE_2. This dysfunctional state reflects the cytokine milieu of AERD (Fig. 1).

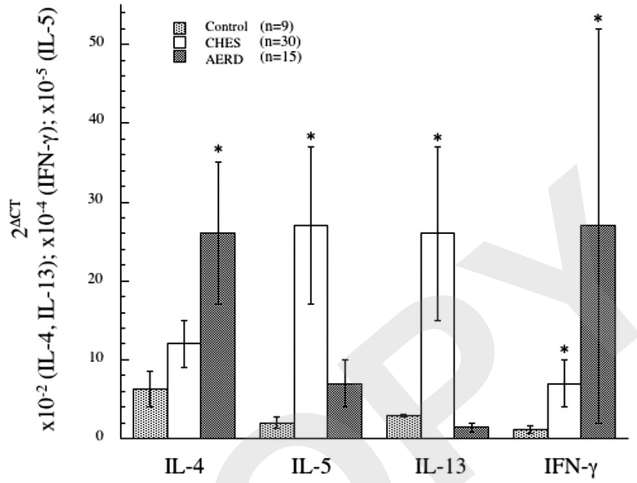

*Figure 2. Th1/Th2 cytokine signature in control, CHES, and AERD sinus tissue by qPCR. Tissue samples were homogenized after surgical removal and RNA isolated. Transcript levels for IL-4, IL-5, IL-13, and IFN-γ were quantified using PCR with SYBR-green detection. Data (mean ± SEM) reflect relative expression of each gene in comparison with β-actin ($2^{\Delta CT}$). Control samples (n = 9) are depicted in black bars, CHES (n = 30) in gray bars and AERD (n = 15) in white bars. *$p < 0.05$ as compared with control. Reprinted from Steinke et al.,[52] with permission from Elsevier.*

CYTOKINE EXPRESSION IN AERD

Numerous studies have addressed the cytokine milieu of eosinophilic sinusitis and, to a lesser extent, AERD. Not surprisingly, most of these studies have demonstrated a prominent T helper (Th)2-like profile, as would be expected in any eosinophilic process.[42–48] However, there are numerous observations that suggest that in contrast to asthmatic or eosinophilic sinusitis patients who tolerate aspirin, AERD seems much more to be a mixed Th2- and Th1-like milieu with prominent expression of interferon (IFN)-γ. This was first suggested in studies demonstrating enhanced IFN-γ expression in a group labeled "nonallergic" sinusitis patients. One characteristic of this group was a higher prevalence of AERD in comparison with the other sinusitis groups.[49] Other investigators have also described high IFN-γ levels in "chronic sinusitis" although, again, did not specify the extent to which this was driven by inclusion of AERD subjects.[50] More recently, a group of adult-onset severe asthmatics with hypereosinophilia and absence of allergy, a cohort in whom AERD subjects were known to be particularly overexpressed, were distinctly characterized by their expression of a high IFN-γ gene signature (Sally Wenzel, AAAAI National Meeting, San Diego, CA; March 2014). The only other study that evaluated IFN-γ expression in AERD demonstrated enhanced levels of IFN-γ in circulating $CD8^+$ cells.[51]

The concept that AERD might reflect a prominent Th1-like component was confirmed in our recently published studies, in which nasal polyp tissue derived from AERD subjects was contrasted from those obtained from aspirin tolerant and control subjects by their overexpression of IFN-γ mRNA transcripts (Fig. 2) and protein.[52] Surprisingly, we subsequently also demonstrated that eosinophils themselves were the most important source for this cytokine[52] consistent with recognition that IFN-γ can be expressed by eosinophils in substantial amounts.[53–55] Eosinophils secrete numerous cytokines and chemokines,[54] and unlike lymphocytes, eosinophils store these cytokines preformed within granules that can be instantly released with activation.[55,56] The robust expression of interleukin (IL)-4 in AERD did not extent to IL-5 (Fig. 2), likely representing the physiology of eosinophils, specifically that IL-4 but not IL-5 is a prominent eosinophil-derived cytokine. Given the capacity of IFN-γ to block IgE class switch recombination, it is intriguing to speculate that this coexpres-

sion of IFN-γ contributes to the frequent absence of allergy in AERD previously noted,[3,5] despite the observed expression of IL-4. The recognition of AERD as a combined Th2-/Th1-like disease prompted us to query the role of this mixed cytokine milieu in driving the AERD phenotype.

HYPEREOSINOPHILIA IN AERD

AERD sinonasal and lung tissue are characterized by dramatic (~10-fold) up-regulation in the number of infiltrating eosinophils.[6,22] An IFN-γ-induced transcription factor (interferon consensus sequence binding protein) can drive the differentiation of eosinophils,[57] leading us to speculate on its role in contributing to this eosinophilia. Consistent with this, we were struck by the capacity of IFN-γ, acting in synergy with IL-5, to promote both the survival and differentiation of mature bilobed, CC chemokine receptor 3- and sialic acid-binding immunoglobulin-like lectin 8-expressing eosinophils from $CD34^+$ hematopoietic progenitors,[52] confirming earlier studies[58] regarding the influence of this cytokine on eosinophil-mediated inflammation. However, for the remainder of this review, we will primarily focus on influences of cytokines on CysLT and PG pathways.

CYTOKINE DYSREGULATION OF LTC_4S AND CysLT RECEPTORS

Mast cells typically express modest levels of LTC_4S and its up-regulation can be mediated by IL-4 (but not by IL-5 or IL-13).[59] Interestingly, a role for IFN-γ in up-regulating LTC_4S expression in umbilical cord-derived mast cell progenitors was also observed (J. Boyce, Harvard Medical School, 2014, personal communications). However, as noted,[22] studies investigating the source of CysLTs in AERD have suggested that eosinophils are likely the more important cell type overexpressing LTC_4S. In our studies, we were unable to demonstrate an ability of numerous innate or adaptive cytokines, including IL-3, IL-4, IL-5, granulocyte-macrophage colony-stimulating factor, IL-1, tumor necrosis factor-α, or even IFN-γ to modulate LTC_4S expression in circulating eosinophils, presumably reflecting their terminal differentiation state. And our studies also failed to demonstrate an influence of IL-4 on LTC_4S expression by eosinophils differentiated from progenitors in the presence of IL-3 and IL-5. In contrast, we demonstrated a significant increase in LTC_4S expression by de novo IFN-γ-differentiated eosinophils.[52] Most importantly, this translated into increased capacity of these newly differentiated eosinophils to secrete CysLTs upon activation. Thus, IFN-γ at present uniquely carries the capacity to drive this disease-defining characteristic of AERD.

As with LTC_4S expression, the expression of the CysLT receptors is regulated by cytokines, including, most prominently, by IL-4 and IFN-γ. IL-4 increases expression of CysLT1 on mast cells[60,61] and monocytes.[62] In our studies, IL-4 also increased the expression of CysLT1 on T and B lymphocytes and eosinophils.[63] Interestingly, our studies also demonstrated robust up-regulation of CysLT1 receptor in response to IFN-γ on T cells and eosinophils.[63] This was an effect that, at the time of publication, we did not appreciate in regard to its likely relevance to AERD. More recently, we have also extended this capacity of IFN-γ to up-regulate CysLT1 receptors to eosinophils newly differentiated from $CD34^+$ progenitors.[52]

CYTOKINE DYSREGULATION OF PGE_2 SYNTHESIS AND EP_2 RECEPTORS

We investigated the molecular mechanism underlying inhibition of PGE_2 synthesis pathways in AERD. For these studies we focused on influences of IL-4, reflecting again, its prominent expression in AERD, its previously described influences on the prostaglandin metabolic pathways,[64,65] and its involvement in the other facets of arachidonate dysregulation previously discussed. IFN-γ is unlikely to be involved in this aspect of the AERD phenotype, given its established role in up-regulating inducible COX (COX-2). Our studies were performed on nasal polyp-derived fibroblasts and mononuclear phagocytic cells. Monocytes were used both as representative inflammatory cells, but also because PGE_2 is their dominant prostaglandin product. Significant inhibition of COX-2 and mPGES-1 (but not COX-1) mRNA and protein expression was observed in response to IL-4.[41] This appears to be a generalized effect of IL-4 insofar as similar inhibition was also observed in fibroblasts. Inhibition of COX-2 and mPGES-1 synergize to result in dramatically less stimulated PGE_2 secretion by monocytes.[41] Thus, in addition to up-regulating CysLT pathways, IL-4 contributes to the AERD phenotype by inhibiting their PG pathways.

However, it is necessary to remark that more than just loss of the tempering influences of PGE_2 underlies these reactions, otherwise all asthmatics and, indeed, even healthy subjects would react to aspirin/NSAID ingestion with activation of their mast cells and eosinophils. We therefore questioned the capacity of aspirin (and other NSAIDs) to directly drive the activation of eosinophils and mast cells.

DIRECT ACTIVATION OF EOSINOPHILS AND MAST CELLS BY ASPIRIN

How aspirin triggers these non-IgE-mediated reactions has been an enigma. We evaluated the capacity of aspirin and other NSAIDs to directly activate eosinophils and mast cells. For these studies, we used the water-soluble aspirin-like compound lysine aspirin (LysASA). Both eosinophils and mast cells displayed Ca^{+2} fluxes after stimulation with LysASA,[66] and similar results were observed with eosinophil eosinophil-derived neurotoxin secretion. Similar results were obtained with ketorolac but not sodium salicylates. To our surprise, when eosinophils from control, aspirin tolerant, and aspirin intolerant subjects were compared, no differences were observed. We suspect the explanation as to why hypersensitivity reactions to aspirin/NSAID are not observed in these control cohorts reflects the relative implication of COX inhibition. As previously discussed, PGE_2 acts through the antiinflammatory EP2 receptor to prevent the acute reactions to aspirin/NSAIDs.[17,37] However, with the diminished expression of and responsiveness to PGE_2 in AERD,[12,14,67] these subjects exist on the precipice of cellular activation, such that even a modest decrease in PGE_2 can have the observed catastrophic effects. The baseline expression and persistence of, by comparison, much higher levels of PGE_2 protects these non-AERD subjects from cellular activation. This model is consistent with recent observations that in a murine model of AERD, knockout of the rate-limiting enzyme responsible for PGE_2 synthesis, specifically mPGES-1, renders these mice susceptible to anaphylactoid reactions after exposure to aspirin.[68]

A further explanation for lack of reactivity in the control cohorts is that a large component of the anaphylactoid response to aspirin is driven by CysLTs (as shown as previously mentioned by the ability of LT modifiers to greatly attenuate their severity[32,33]). Interestingly, we never observed LysASA or ketorolac-mediated CysLT secretion from circulating eosinophils, even when obtained from AERD donors. AERD sinonasal and lung tissue is characterized by high numbers of eosinophilic hematopoietic progenitor ($CD34^+IL\text{-}5R\alpha^+$) cells[69,70] that will mature in the presence of IFN-γ and, as previously discussed, acquire the ability to produce CysLTs. This would suggest that the robust CysLT production would be limited to airway, and not circulating, eosinophils. We therefore investigated whether eosinophils differentiated from progenitor cells in the presence of IFN-γ would recapitulate the sensitivity to aspirin displayed by tissue eosinophils in vivo in AERD. Consistent with their increased LTC_4S expression, CysLT secretion was subsequently detected upon LysASA activation (Fig. 3).[66]

ASPIRIN DESENSITIZATION IN AERD, TARGETING OF IL-4

Aspirin desensitization is an effective treatment for AERD and has been associated with the diminished need for nasal endoscopic sur-

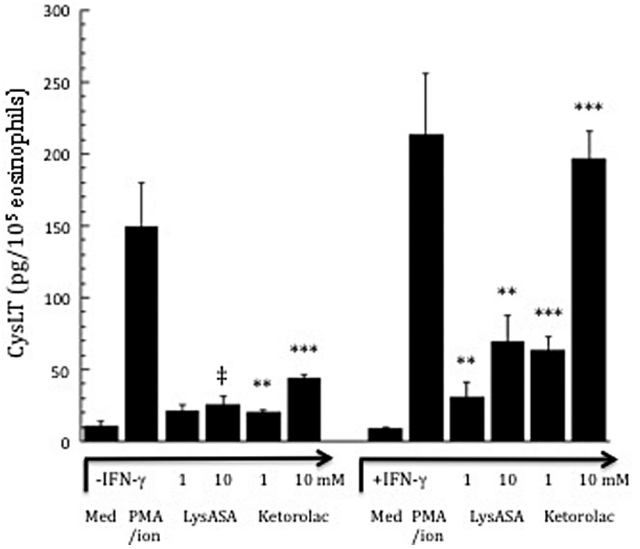

*Figure 3. Activation of eosinophils differentiated from hematopoietic precursors in the additional presence of IFN-γ. CD34$^+$-enriched hematopoietic progenitor cells were cultured for three days with SCF, TPO, Flt3L, IL-3, and IL-5, after which they were cultured for three additional weeks with just IL-3 and IL-5 with or without the additional presence of IFN-γ. Eosinophils matured from CD34$^+$ progenitors were activated with or without calcium ionophore/PMA, LysASA (1mM and 10mM) or ketorolac (1mM and 10mM) for 30 minutes. Supernatants were collected and CysLT levels quantified and measured in pg/mL. Data are presented as the mean ± SEM of a minimum of six separate studies. ‡$p = 0.05$, **$p < 0.03$, ***$p < 0.002$ as compared with unstimulated cells. Reprinted with permission of the Journal of Immunology. Source: Reference 66.*

gery, improved sense of smell, fewer bouts of acute sinusitis, reduced need for oral corticosteroids, and less severe asthma.[2,71,72] The molecular mechanisms driving the beneficial effects of aspirin have not been determined, but we believe it is due in part to the ability of this compound to inhibit the biologic activities of IL-4 (and perhaps, by extension, IFN-γ). Consistent with this concept are the observations that successful aspirin desensitization is associated with reversal of many of the IL-4-modulated features of AERD discussed above, including the ability of desensitization to down regulate CysLT synthesis and responsiveness pathways.[23,24,73] There are many mechanisms by which aspirin may induce these effects. However, we focused on recognition that engagement of the IL-4 receptor by IL-4 induces activation of signal transducer and activator of transcription (STAT)6. A STAT6 site in the CysLT1 receptor promoter is central to its IL-4-mediated transcription, and similarly, a putative STAT site has been identified in the LTC_4S gene.[74] Aspirin inhibits the activation of STAT6,[75] suggesting to us that aspirin may produce its clinical utility in AERD through direct inhibition of the IL-4-activated STAT6 pathway. Our studies investigated the inhibition by aspirin and other NSAIDs of the STAT6-mediated regulation of the CysLT1 receptor and LTC_4S genes.[41] In a dose-dependent fashion, aspirin inhibited transcription of IL-4-induced CysLT1 receptors. Subsequently, via electrophoretic mobility shift, oligomer competition, and supershift assays, we confirmed the presence of STAT6-binding sites within the CysLT1R and LTC_4S promoters. Both by electrophoretic mobility shift and Western hybridization assays, our data demonstrated the absence of phosphoSTAT6 protein within the nuclei of aspirin-treated cells (Fig. 4). These results were extended to other NSAIDs, including ketorolac, but not sodium salicylate. The mechanism by which aspirin blocks pSTAT6 nuclear expression is not known but has been suggested to involve nuclear trafficking and recycling of transcription factors.[76] These data suggest that aspirin desensitization may provide

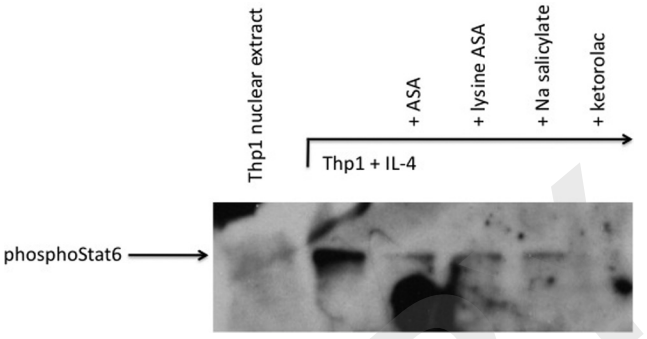

Figure 4. Western hybridization of nuclear extracts. Nuclear extract proteins were separated on a SDS polyacrylamide gel and transferred to a nitrocellulose membrane. Presence of phosphoStat6 was determined via probing with an anti-phosphoStat6 antibody and chemiluminescent detection. Reprinted from Steinke et al.,[41] with permission from Elsevier.

effective therapy for AERD, in part through mitigation of STAT6 activation, leading to down-regulation of the leukotriene pathways. That similar mechanisms could be involved in blocking IFN-γ-activated pathways (*e.g.*, STAT1 trafficking) would be an intriguing area for future research.

SUMMARY

Toward a Generalized Model for the Induction of the AERD Phenotype

Although the exact mechanisms driving AERD are not fully understood, part of the explanation is the marked overexpression of the 5-lipoxygenase and LTC_4S genes, resulting in constitutive overproduction of CysLTs, and the decrease in PGE_2 expression that would normally act to constrain mast cell and eosinophil activation. We present a series of studies that strongly suggest that this AERD phenotype is derived, in large part, from the increased expression of both IL-4 and IFN-γ and that, as such, this disease reflects a mixed Th1/Th2 process. The increased expression of proinflammatory mediators and loss of protective PGE_2 leads to uncontrolled release of mediators, and especially CysLTs, when eosinophils and mast cells are directly trigged by aspirin in AERD subjects. This increased understanding of the cellular reactions provides an opportunity to develop new therapeutic approaches aimed at dampening the severe impacts of this disease.

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