REVIEW ARTICLE

Aspirin-induced asthma: a still evolving area of basic and clinical research

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ABSTRACT

The first modern description of respiratory syndrome of aspirin hypersensitivity was published over half of the century ago, but the pathogenesis of the disease is still elusive. Just a few years after discovery how aspirin works, Andrew Szczeklik and his co-workers described that asthmatics with aspirin hypersensitivity cross-react to the whole class of nonsteroidal anti-inflammatory drugs. It took rest of his life to seek for an answer on how this disease, nowadays referred to as N-ERD, develops and how it can be treated. In the meantime, cysteinyl leukotrienes, leukotriene modifying drugs, and novel subpopulations of lymphocytes were discovered. This review on aspirin hypersensitivity documents a progress in our understanding of mechanisms of hypersensitivity to nonsteroidal anti-inflammatory drugs. Current concepts about origin of the disease integrate advances in the field of allergology and inflammatory mechanisms of asthma. However, pharmacological inhibition of prostaglandin biosynthesis by nonsteroidal anti-inflammatory drugs has a pivotal role in these investigations. Presented is a central role of prostaglandin E_2, a double-faced lipid immunoregulatory mediator whose deficiency is related to the administration of an anti-inflammatory drug. Discussed are cysteinyl leukotrienes, the most reliable biomarkers of aspirin hypersensitivity and cells of innate immunity capable of leukotrienes production. Involvement of blood platelets and recently described mucosal basophils are areas of ongoing studies in the disease. Aspirin hypersensitivity is an acquired condition; therefore, the search for genetic predisposition using classic association studies was inconclusive. There is a new hope to explain mechanisms of aspirin hypersensitivity by studies of innate lymphoid cells, which have a central role in the regulation of respiratory mucosa function in asthma.

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History of aspirin hypersensitivity In 1897, Felix Hoffmann, a German chemist employed by the Bayer company, successfully produced acetyl ester of salicylic acid—acetylsalicylic acid (ASA). He noted in his lab book that it "differs favorably from salicylic acid in its physical characteristics, such as sour taste without any corrosive effect, and is therefore being tested for its usability in this context." After first clinical trials in 1898, ASA was found to be effective against pain, inflammation, and fever. The Bayer company decided to market acetylsalicylic acid under the name of Aspirin. The etymology of this name was *A* as in *acetyl* and *spiric* as in *Spiraea ulmaria*, a common meadowsweet herb. The original Bayer Aspirin tablet had a content of 324 mg of ASA. In 1902, there was the first clinical observation by Hirschberg in Poznan of a transient angioedema and urticaria in a patient after ASA ingestion. In 1919 and 1920, reports by Cooke and Van der Veer documented acute bronchospasm or fatal asphyxia several minutes after ingestion of ASA. Fernand Widal described association of ASA idiosyncrasy with asthma and nasal polyps in 1922. The name of aspirin triad was proposed Samters and Beers in 1968 for a respiratory syndrome of chronic rhinosinusitis, asthma, and ASA hypersensitivity.

Respiratory symptoms of ASA hypersensitivity Samter's triad or aspirin asthma eventually became obsolete names of the disease. In the United States, the term aspirin-exacerbated respiratory disease is still in use. More recently, a name nonsteroidal anti-inflammatory drugs (NSAIDs)–exacerbated respiratory disease (N-ERD) was promoted by the European

Academy of Asthma and Clinical Immunology.[1] The disease is fairly common, estimated to affect from 5% to 15% of asthmatics.[2-6] Within the subgroup of severe asthma, the prevalence of N-ERD is up to 25%; if chronic rhinosinusitis and nasal polyposis are present, N-ERD can be diagnosed in 40% asthmatics. In the general population the prevalence is above 0.5%, varying across the studies. Hypersensitivity to ASA is much less frequent in children but in childhood asthma refractory to treatment it approaches 30%.[7,8] The classic course of the disease is characterized by initial presence of chronic rhinosinusitis resembling a persistent viral infection and accompanied by a graduate loss of smell.[9] Subsequently, nasal polyposis develops, next accompanied by symptoms of asthma. The hallmark of N-NERD are episodes of violent bronchoconstriction after ingestion of aspirin or other NSAIDs. However, these drugs are frequently self-administered as pain-killers and are available over the counter under different trade names. Thus, about 15% of asthmatics remain unaware of their NSAID intolerance until diagnosed using a drug challenge tests.[9] Therefore, it is more credible to exclude N-ERD if an asthmatic patient reports frequent use of NSAIDs without adverse reactions. A benchmark diagnostics requires an NSAID provocation test, performed in the clinical setting to ensure the patient's security.[1,10,11] This is required because some patients respond to the drug challenge with a violent eruption of symptoms. The second reason is that after a positive provocation test with NSAIDs, a follow-up is necessary due to the late asthmatic response in some N-ERD patients. There are different NSAID provocation procedures proposed,[12,13] but the most recommended by specialists is the oral challenge test, which based on administration of incremental doses of aspirin given every 90 minutes over 6 to 7.5 hours. If the cumulative dose reaches 1000 mg without any symptoms, patient is deemed aspirin-tolerant and N-ERD is excluded.[14] In the United States, oral provocation with ASA is usually split over 2 days. As with other provocative test used in allergology, the previous-day placebo trial should precede the drug challenge to ensure a stable condition of asthma. During the challenge, pulmonary function tests (PFTs) are repeatedly performed before each dose of ASA and 30 minutes thereafter. A decrease in forced expiratory volume by 20% indicates a positive test and stops the provocation procedure. The resulting bronchoconstriction has to be reversed by β-mimetics. A substantial fraction of N-ERD or N-IUA patients have a blended reaction affecting more than one organ.[15] It often manifests as a simultaneous appearance of pulmonary and extrapulmonary symptoms during the drug challenge, accompanying PFT deterioration. The most common extrapulmonary signs are itching of conjunctiva and the nose, nasal discharge, and flushed skin of the neck. Also, angioedema of lips and oral mucosa, harsh voice, and a typical urticaria eruption are sometimes observed. A response to the provocation with ASA without bronchoconstriction is characteristic to the other manifestation of ASA hypersensitivity, that is, NSAIDs-induced urticaria angioedema (N-IUA) or NSAIDs exacerbated chronic idiopathic urticaria. A particular cardiological manifestation of an acute coronary syndrome during the drug challenge or symptomatic exacerbation of N-ERD is named Kunis–Zavras syndrome.[16]

Pathogenesis of ASA hypersensitivity remains a subject of clinical research. The first theories were focused on drug allergy.[17,18] Patients sensitized to particular drugs are diagnosed by topical provocation tests, that is, intradermal injection of the culprit drug or, less frequently, by a drug skin patch test. A typical sequence of local symptoms develops for either type I mediated allergy or type IV delayed response, according to the Gell and Coombs classification of drug allergies. There are plenty data published about immune-based hypersensitivity to NSAIDs. Pyrazolones (eg, metamizole)[19] and arylpropionates (eg, ibuprofen)[20,21] are among the most commonly sensitizing drugs. However, patients with immune-based hypersensitivity do not cross-react, when tested, with other chemical classes of NSAIDs (eg, ASA).

Cyclooxygenase theory of N-ERD and N-IUA A cross-reactivity to the drugs representing different chemical classes is a distinct feature of N-ERD and N-IUA. Discovery of cyclooxygenase and inhibition of prostaglandin synthesis[22] explained the common pharmacological mechanism of NSAID activity. Cyclooxygenase is a highly specific enzyme oxidizing arachidonic acid, an abundant constituent of biological membranes of the cell. The enzyme requires as a substrate free arachidonic acid released from biological membranes by calcium-dependent phospholipases. An intermediary product of this oxidation, endoperoxide named prostaglandin G_2, is converted by the same enzyme into prostaglandin H_2 (PGH_2). NSAIDs can mimic arachidonic acid structure and block the substrate pocket of cyclooxygenase. Biologically active prostaglandins are products of downstream enzymatic isomerization of PGH_2 by specific isomerases, also called synthases. Practically, each cell of the body can produce prostaglandin E_2 (PGE_2), whereas some cells express also synthases of prostaglandins D_2 or F_2. Therefore, the potency of NSAIDs is defined as the inhibitory activity of the drug on prostaglandin synthesis. This concept was conceived and confirmed for the first time in 1975 by Szczeklik, Gryglewski and Czerniawska-Mysik.[23-25] An unusual clinical finding of asthmatics cross-reacting to different NSAIDs was explained by inhibition of PGE_2 biosynthesis in vitro using microsomal fraction of a tissue. A common feature of different NSAIDs is their inhibitory activity against cyclooxygenase. However,

when a second isoenzyme of cyclooxygenase was discovered (COX-2),[26] more data on the preferential inhibition of COX-1 versus COX-2 were collected. It turned out that N-ERD patients tolerate selective COX-2 inhibitors, whilst a drug potency against COX-1 isoenzyme is inversely correlated with the minimal dose of drug precipitating symptoms of hypersensitivity.[27] There are not many selective or highly specific COX-2 inhibitors available on the pharmacy market. Rofecoxib was retracted, but celecoxib, etoricoxib, and lumiracoxib are available. These drugs are tolerated by N-ERD or N-IUA patients if administered in regular doses,[28] because coxibs inhibit COX-1 much less potently than COX-2. Conversely, NSAIDs available without prescription, including ASA, are nonselective cyclooxygenase inhibitors. Some consequences of the cyclooxygenase theory of N-ERD, as originally proposed by Szczeklik et al,[29] were thoroughly investigated in the following years. Clinical trials showed that PGE_2 inhalation can prevent bronchoconstriction during the NSAID challenge. Unfortunately, this prostaglandin evokes a cough reflex by stimulation of sensory fibers in the airways. This led to a detailed analysis of cell signaling by PGE_2. Prostaglandins D_2 and F_2 are, in general, proinflammatory mediators. However, PGE_2 has a dual physiological role. Depending on the type of its receptor, PGE_2 can either increase intracellular cyclic adenosine monophosphate or inhibit cytosolic adenylate cyclase, or even signal through intracellular calcium release along the inositol triphosphate pathway.[30] During typical inflammation, for example, bacterial infection, inducible COX-2 increases manifold the production of PGE_2, which results in inflammatory responses like fever, enhanced bacterial phagocytosis, and edema. However, 2 PGE_2 receptors, EP2 and EP4, are coupled to the adenylate synthase by a stimulatory Gα subunit of the receptor. EP2 can stabilize innate immunity cells and prevent release of their proinflammatory mediators.[31] It was documented that circulating granulocytes of N-ERD patients are defective for this anti-inflammatory property of EP2 signaling.[32] Moreover, the only animal model of N-ERD studied so far is a mice knock-out strain deficient in PGE_2 microsomal synthase type-1.[33] It is highly interesting that, despite the uniform distribution of the transcript of the *PGES* gene encoding this synthase across different cell types, the highest protein level was documented in the respiratory epithelium.[34] Direct comparisons of *PGES* mRNA expression in respiratory epithelia or bronchial fibroblasts obtained by bronchoscopy of N-ERD and ASA-tolerant patients documented decreased transcription of the gene in the airways of ASA-hypersensitive individuals. Similar findings were documented in explants from nasal polyps of N-ERD patients.[35] On the receptor level, antiphlogistic activity of PGE_2 in the airways seems also lessened, because the EP2 receptor expression was found to be reduced in N-ERD patients.[36]

Cysteinyl leukotrienes and aspirin hypersensitivity

The existence of a potent mediator different from histamine but causing bronchoconstriction was demonstrated by Feldberg and Kellaway in 1938 and 1940. A descriptive name of "slow-reacting substance of anaphylaxis" was used for this substance released by a phospholipase from cobra venom, but also during anaphylactic shock in a sensitized guinea pig lung. In 1979, Samuelsson characterized the chemical formula of the mediator and suggested a name of leukotriene.[37] Cysteinyl leukotrienes are proinflammatory mediators produced natively by mast cells, basophils, eosinophils, or by intercellular metabolism between granulocytes and platelets. Their biosynthesis requires the activity of arachidonic acid 5-lipoxygenase (5-LO) and subsequent glutathione S-transferase. The initial glutathione conjugate is leukotriene C_4 (LTC_4), a gamma-glutamyl residue quickly trimmed off by cell surface proteases to form leukotriene D_4, next converted to leukotriene E_4 (LTE_4) by a peptidase removal of glycine. LTE_4 is a stable compound, in which cysteine is bound by a sulphur atom to 5-hydroxy arachidonic acid (5-hydroxytetraenoic acid [5-HETE]). Cysteinyl leukotrienes contract bronchial smooth muscles at a 1000-fold lower molar concentration than histamine. Enzymatic steps tightly control the biosynthesis of cysteinyl leukotrienes. The first step is the release of arachidonic acid by a phospholipase A2 from the intracellular membranes, the second is activation of 5-LO which requires adaptor protein 5-lipoxygenase–activating protein. The ultimate step is catalyzed by LTC4 synthase, a specialized glutathione S-transferase expressed only in limited cell populations. N-ERD patients overproduce cysteinyl leukotrienes,[38,39] which can be conveniently measured as urinary LTE_4 excretion. During NSAID provocation or exacerbation of the disease, excretion of LTE_4 in the urine can rise severalfold.[40-42] It correlates well with bronchoconstriction measured by spirometry and returns to the baseline excretion within a few hours after respiratory symptoms subside. Cysteinyl leukotrienes stimulate their receptors of type-1 (CysLTR1) and type-2 (CysLTR2). Respiratory symptoms of N-ERD are mediated by both of the receptors. CysLTR1 contracts bronchial smooth muscle cells, increases vascular permeability causing edema, stimulates bronchial secretions, and is chemoattractant for inflammatory cells, enhancing their proliferation.[43] CystLTR2 is far less studied but expressed on neural dorsal root ganglia, where stimulation of the receptor causes itching. It also activates platelets and angiogenesis, participating in the eosinophilic inflammation of the lung along with interleukin (IL) 33 produced by epithelial cells.[44,45] Pretreatment with highly effective CysLTR1 antagonists (monelukast, pranlukast) does not protect against NSAID-induced respiratory reactions. Moreover, clinical benefits from CysLTR1 inhibition in N-ERD are rather limited.[46,47] Thus, some researchers believe that respiratory pathology of

N-ERD is mediated by CysLTR2, whereas others postulate the existence of a third receptor of cysteinyl leukotrienes. Some candidates for CysLTR3 are G-protein coupled receptor GPR99, known as the oxoglutarate receptor[48-50] and GPR17.[51,52] N-ERD patients reacts to inhalatory provocation with LTE_4, whose leukotriene has the lowest affinity to CysLTR1 or CysLTR2. Therefore, studies on alternate LTE_4 receptor–mediating bronchoconstriction in N-ERD are still needed.

Genetic predisposition to N-ERD and N-IUA Human genome accommodates about 3 million single nucleotide variants of DNA sequence. Some of these variants do not change the amino-acid sequence of the encoded proteins or these single nucleotide polymorphisms (SNPs) do not have obvious functional consequences. Other SNPs are located within regulatory domains of DNA and potentially can affect the genetic expression by altered transcription rate. Studies on genetic predisposition to NSAID hypersensitivity focused on genes of the immune system and the encoding proteins involved in the signaling of lipid mediators. Since familial cases of N-ERD or N-IUA are rare, research on genetics was conducted using a contrast between aspirin-hypersensitive patients and aspirin-tolerant patients or healthy controls. The first signal of association was found between N-ERD and HLA-DRb locus,[53-56] and was replicated in different ethnicities. However, consequences of this association with the major histocompatibility complex gene remains speculative. Other positive association signals included a common LTC4S promoter polymorphisms, SNP variants of the *FLAP*, *CysLTR1* and *CysLT2*, *EP2*, and other genes[57-68] whose products participate in the signaling of prostaglandins or leukotrienes. It is interesting that no consistent genetic association signals were found for either cyclooxygenase gene. Most of these studies reported findings in a limited number of patients and were not replicated, perhaps with the exception of the LTC4S association, also noted in N-IUA.[69,70] Another problem of genetic studies is a variable phenotype of N-ERD patients, which introduces a recruitment bias into genetics studies.

The role of platelets in aspirin hypersensitivity During an acute allergic reaction, blood platelets are activated. This was documented well for allergen-induced bronchoconstriction. However, in aspirin hypersensitivity this phenomenon does not seem secondary to platelet activation by inflammatory mediators released from the mast cell. Circulating aggregates of platelets adhering to granulocytes were measured in the peripheral blood of N-ERD patients and their number was elevated despite good asthma control.[33,71-75] During a challenge test, the number of aggregates rose quickly. Platelets express the LTC4S enzyme but cannot produce 5-HETE. Adherence of platelets to granulocytes enables the production of cysteinyl leukotrienes by transcellular diffusion of 5-HETE from these cells. Activation of granulocytes normally results in the release of a neutrophil chemoattractant leukotriene B4, a product of 5-LO spontaneously formed by the cells expressing the enzyme but lacking LTC4S activity. A clinical trial revealed that inhibition of platelets using irreversible inhibitor of $P2Y_{12}$ platelet receptor indeed decreased urinary excretion of LTE_4 and ameliorated asthma symptoms in some N-ERD patients.[76] Thus, inhibition of platelet activity can improve the course of the disease, but only is some patients.

Mast cells as a source of mediators in N-ERD and N-IUA Inflammatory mediators were measured in N-ERD patients not only in the urine (LTE_4) but also in the peripheral blood, nasal lavage fluid, bronchial lavage fluid, and in the condensate of exhaled air.[42,77-82] In all these materials, the level of CysLTs increased following NSAID provocation. Since the provocation procedure is performed with a cyclooxygenase inhibitor, it is difficult to demonstrate changes in PGE_2 levels accompanying the oral provocation test. Because prostaglandin is quickly inactivated in the circulation, measurements of urinary metabolites of PGE_2 showed a decrease during the drug challenge in aspirin-tolerant asthmatics, but remained unchanged in N-ERD patients following aspirin administration.[83] It required a second challenge with the preferential COX-2 inhibitor, celecoxib, to show that about 40% of systemic production of PGE_2 is derived from the COX-2 enzyme activity. Apparently, the decrease in the PGE_2 produced by COX-2 does not contribute to the precipitation of bronchoconstriction. It is the lack of the PGE_2 produced by COX-1 that incites N-ERD symptoms. Mast cells are highly reactive immunocytes, which normally degranulate following binding of an allergen to their FCeR1-IgE receptor complex. Histamine released during degranulation causes the majority of mucosal or skin symptoms and participates in the airway inflammation. However, mast cells not only release the preformed mediator, but also produce hematopoietic or inflammatory cytokines and lipid mediators. A practical marker of mast cell activity is prostaglandin D_2 (PGD_2). Increased levels of histamine and tryptase released during degranulation,[84,85] along with PGD_2, were well documented during a drug challenge test in N-ERD.[12,81,82,86] The contribution of mast cells to systemic production of CysLTs is well confirmed, but this is not the sole source of leukotrienes. Mast cells are involved in all immediate allergic reactions; perhaps this is why studies on their role in the pathogenesis of N-ERD and N-IUD are not promising. Cromones, drugs known to stabilize the mast cell against activation and degranulation, can temporarily improve NSAID tolerance, for example, during the oral ASA challenge,[87] but there are no other benefits of cromone therapy in patients receiving NSAIDs. Despite this fact, basophils, whose receptors and mediators are similar

to mast cells, attracted attention in N-ERD for 2 reasons. First, a laboratory assay of basophil activation was tested for discerning aspirin hypersensitivity in vitro with discouraging results.[88] Second, immunohistochemical staining of basophil lineage markers documented the presence of these cells in the mucosa of N-ERD patients, but not in aspirin-tolerant asthmatics.[89] This is a relatively new discovery, and it is not yet known how it contributes to our understanding of the N-ERD pathogenesis.

Eosinophils and eosinophilia in N-ERD The peripheral blood eosinophil count is usually higher in N-ERD patients compared with in aspirin-tolerant asthmatics. However, this laboratory marker lacks any specificity. Eosinophilic inflammation of the mucosa is a frequent finding in allergic disorders; therefore, it is not specific to N-ERD. Nasal polyps, a common manifestation of N-ERD, are also accompanying chronic rhinosinusitis with or without asthma.[90] The key cytokine responsible for an elevated number of circulating eosinophils, their migration into tissues, activation, and prolonged life span is IL-5. Despite eosinophilic inflammation, the blood level of IL-5 in N-ERD patients is not elevated.[91] A highly effective biological therapy with mepolizumab, a monoclonal anti–IL-5 IgG does not improve the clinical course of N-ERD.[92,93] It is therefore plausible that eosinophils are effector cells, whose activation can be documented during a hypersensitivity reaction to NSAIDs, but these cells are not responsible for the pathogenesis of N-ERD. However, there are some interesting findings published on eosinophils collected from the respiratory tract in N-ERD. These cell have additional properties, such as a constant expression of LTC4S, production of interferon γ and PGD_2, and overall sensitivity to NSAIDs, which, in a dose-dependent manner, stimulate the release of mediators.[94]

Immunological dysregulation as a mechanism of aspirin hypersensitivity was proposed only recently. About a decade ago, new subpopulations of innate-immunity lymphoid cells were identified. These cells are different from the subpopulations generated during an adaptive immune response because they do not switch through a somatic recombination their receptor or immunoglobin chain region. Innate lymphoid cells (ILC) are invariant and do not express B- or T-cell immunoreceptors at all. However, their phenotype can mimic that of T-lymphocytes owing to a capacity to produce mediators similar to Th1 or Th2 subsets of T-helper cells. These cells are tissue-resident and especially numerous in the mucosal membranes.The ILC2 subset has CysLTR1 and DP receptors for cysteinyl leukotrienes and PGD_2.[95] These lipid mediators are potent chemoattractants for ILC2. Major stimulants of these cells are thymic stromal lymphopoietin (TSPL) and other cytokines produced by the respiratory epithelium in response to pathogens such as IL-33 and IL-25.[96] Stimulated ILC2 cells can produce large quantities of type-2 cytokines, including IL-4, -5 and -13, characteristic for allergic inflammation. It was demonstrated that during aspirin challenge, ILC2 cells accumulate in the respiratory mucosa and contribute to the release of inflammatory mediators.[97]

A possibility of chronic infection participating in the pathogenesis of N-ERD was also investigated. In some studies, an elevated level of IgE against *Staphylococcus aureus* endotoxin superantigens was described.[98,99] This finding is also common in asthmatic patients with chronic rhinosinusitis and those with nasal polyposis. Another tempting hypothesis studied was a persistent viral infection of the airways in N-ERD patients. Using a sensitive technique of in-situ amplification, traces of human rhinovirus genome were found more frequently in bronchial mucosa microbiopsies of N-ERD patients.[100]

Further studies on aspirin hypersensitivity are warranted and will focus on abnormalities in the expression of genes characterizing this disease. An impaired respiratory epithelium barrier can implicate an enhanced signaling by TSPL and other cytokines (such as IL-33 and IL-25), cause an enhanced response of the respiratory epithelia to damage-associated molecular patterns, abnormal toll-like receptors signaling, or epigenetic changes caused by hyper- or hypomethylation of the genes in a mechanism of somatic imprinting. Cross-reactivity to chemically different NSAIDs was described by Andrew Szczeklik and co-workers in a small group of patients soon after the discovery of pharmacological inhibition of prostaglandin synthesis. Although it is far more common to see asthmatic patients sensitized to certain environmental or food allergens, or to drugs, a discovery made 5 decades ago inspired many research groups to investigate cellular, molecular, immunological, and genetic mechanisms of N-ERD and N-IUA. And this quest is still ongoing.

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