

New Developments in Basic Biology of Henoch-Schönlein Purpura

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“From its origin, the exanthem spreads over the whole body with the exception of the palms and soles of the feet. Clearly wrong is the notion that only the covered and thus exposed to heat parts of the breast are effected by Friesel, since also it appears as frequently on the neck and in the face. By the way, the development of the exanthem never happens all at once, but always in bouts (immer stossweise.) And that’s characteristic of the Friesel, because other exanthems don’t do that.”

-- Dr. Johann Schönlein, in 1834 lecture notes about “purpura rubra,” Pathologie und Therapie (vol 2, p320.)

When parents bring children with Henoch-Schönlein purpura (HSP) to the emergency room, the pediatric rheumatologist can generally reassure them that greater than 90% will recover completely from their dramatic presentation [1]. But considerable head scratching ensues in response to questions about what precipitates the illness, why it affects primarily the skin, bowel and kidneys, and how one can predict which children will go on to develop renal insufficiency or failure later in life. A set of four generally accepted criteria were posited by John Mills and an American College of Rheumatology study group [2]. They found a sensitivity and specificity of 87% when patients met two or more criteria, namely: non-thrombocytopenic palpable purpura, diffuse post-prandial abdominal pain or bloody diarrhea, onset at age twenty or younger, and PMNs in arteriolar or venular walls on biopsy. Considerable progress was attained in understanding the genetics and pathophysiology of HSP this past year, complementing new work that has identified clinical risk factors for the most severe outcomes [3] [4].

Genetic determinants of susceptibility and severity

“Patients suffer rheumatic affections of the lining of the stomach which creates a constant pressure on the stomach with excruciating pain leading to vomiting.”

One group in Northern Spain published six papers this past year detailing the role that five different genes appear to play in susceptibility to HSP and risk of disease severity. The first two genes reside in different classes of the major histocompatibility complex (MHC). 50 HSP patients (39 children) were found to have an increased incidence of the **DRB1*01 MHC allele** compared with ethnically matched controls [5], although expression of the related phenotype had no impact on disease manifestations or severity. In contrast when they looked at expression of the more telomeric **HLA-B35** gene, there was no difference between patients and controls [6]. However, 10/31 patients with hematuria or proteinuria were B35-positive, compared to none of 17 without renal manifestations. HLA-B35 is thought to lie in linkage disequilibrium with the DRB1*01 locus, but two other similarly linked HLA-B genes were not found to be increased among HSP patients with renal disease [6]. Thus expression of the HLA-B35 allele appears to uniquely predispose a child to the development of renal complications in HSP, independent of any haplotype effects or putative T-helper cell activation that is linked to alleles within the class II DR region of the MHC.

An overlapping group of 52 patients (including 41 children) with at least one year of follow-up was also studied for variations in two common coding region genetic alleles of the **ICAM-1 adhesion molecule**[7], which is highly expressed in glomerular lesions from HSP and other glomerulonephritis patients [8]. One particular variant of the ICAM-1 gene, a substitution of glutamate for lysine as a result of mutation at codon 469 imparted more than a six fold increased risk for GI complications compared to those patients without GI problems. This genetic variant had previously been shown to be twice as common among a cohort of Middle Eastern Behcet's patients, compared with matched controls [9].

Inflammatory factors predisposing to renal disease

“The diseased starts to sweat heavily with a peculiar, disheartening odor like vinegar gone bad...There is a small amount of urine, which also reacts acidic and makes sediment—which consists of rosy uric acid.”

IL1RA

A number of inflammatory diseases in children have been linked to different alleles of a variation in the **IL1 receptor antagonist gene (IL1RA)** on chromosome 2q14-q21. In the ten years since the initial description of variable numbers of tandem repeats (VNTR) inserted into the second intron

of this gene [10], both disease susceptibility and severity have been linked to these alleles. Specifically, several forms of juvenile chronic arthritis are associated with increased incidence of allele 2 (of five that have been described) [11]. Inflammatory myopathy susceptibility has been linked to alleles 1 and 3, depending on ethnicity [12]. Neither the JCA nor the myopathy papers found any differential in disease activity between individuals with the implicated alleles and those patients with the more common alleles. In HSP, the issue had been examined in a Chinese population [13], with no allelic frequency difference identified between patients and controls. However, they did find that allele 2 of the **IL1RA** gene did significantly predispose patients with either HSP or IgA nephropathy to hematuria.

The role that **IL1RA** plays in HSP disease severity was revisited this past year by Amoli *et al.*, who posed the question among the same Spanish population studied for ICAM-1, HLA-B35 and DRB1 allelic variation, as described above. In studying 58 HSP patients (and 38 additional individuals with hypersensitivity vasculitis), they looked for genotypic differences compared with a group of 109 ethnically-matched controls [14]. Their results showed again that disease severity, but not susceptibility, was affected by the **IL1RA** genotype. Among those who developed vasculitis, the **IL1RA** allele 2 was a significant predictor nephrotic syndrome, renal insufficiency, and permanent renal involvement. But in contrast to the earlier work, hematuria (as seen in the Chinese patient group) was not predicted by the **IL1RA** genotype.

Overall, the allelic variation in the second intron of the **IL1RA** gene has been associated with increased severity of nephropathy in HSP, diabetes, and IgA nephropathy; with more severe skin disease in lupus [15], alopecia totalis and universalis [16], lichen sclerosis [17] and early-onset psoriasis [18]; and susceptibility to disease in JCA, juvenile myositis, ankylosing spondylitis [19], Graves' disease [20], and ulcerative colitis [21] compared with normal controls. Interestingly, although allele 2 was associated with severity of disease in a cohort of 81 lupus patients (risk for discoid lesions and photosensitivity), the disease allele did not put these patients at additional risk for renal disease as it did for children with HSP [15].

These alleles are defined by the number of tandem repeats for an 86 base pair sequence within the second intron, thus not involving directly the coding sequence of the gene. The effect of the polymorphism on the biology

of the IL1 system is unclear. Studies on IgA nephropathy patients, including those with HSP complicated by renal disease, have found significantly decreased levels of **IL1RA** in the peripheral circulation [22]. But the previous work showing allelic differences in myopathy patients failed to find any effect on circulating **IL1RA** levels, and gene splicing is apparently not affected by this intronic iteration [12]. **IL1RA** levels in normal individuals typically exceed circulating IL1 levels by greater than 10-fold [22], theoretically tipping the balance toward the non-inflammatory end of the spectrum under homeostatic conditions.

In contrast to the TNF blockers routinely used in clinical practice, which immobilize TNF α itself, **IL1RA** binds mutely to the IL1 receptor and not to the receptor ligands IL1 α and IL1 β . Consequently, production of **IL1RA** at the target tissue may become more important for fine control of the inflammatory cascade. Regulation of **IL1RA** and IL1 gene expression can be induced inversely by pro- and anti-inflammatory stimuli at sites of inflammation [23]. Indeed, HSP patients have been previously shown to have increased levels of IL1 (and TNF α) in their urine compared to patients with other forms of nephritis, and it has been suggested that local production of cytokines may contribute to the mesangial proliferation and loss of filtrative discrimination [24]. Because IL1 is a potent inducer of mesangial proliferation [25], loss of homeostatic control could be a root cause of this element of the renal pathology.

CYTOKINES

The fourth class of genes the Spanish group examined was cytokines, several of which have been implicated in the activation of neutrophils that may be major initiators of endothelial damage in HSP. **Interleukin-8 (IL-8)**, epithelial cell-derived neutrophil-activating peptide (**ENA-78**), and regulated upon activation normal T cell expressed and secreted (**RANTES**) all participate in the recruitment of neutrophils to sites of inflammation. Genotyping for several common alleles of each in 50 HSP patients demonstrated no differences compared with matched controls for any as a susceptibility factor to HSP. However, those individuals with a common IL8 polymorphism appeared somewhat more likely to develop renal disease [26].

At a functional level, other cytokines are undoubtedly important in modulating the microvascular environment at the site of injury. Besides

elevations in circulating TNF α and IL6, HSP patients have increased levels of **vascular endothelial growth factor (VEGF)** during the acute phase of disease [27]. This new study, examining 34 Turkish children with HSP, found no differences in **VEGF** levels relative to the different clinical presentations (*e.g.* gastrointestinal involvement, renal disease). The most interesting elements of the work pertained to time and space. In contrast to many other mediators, which have been shown to reach their acme during acute disease, **VEGF** expression in the vascular wall was maximal in the recuperation phase of the disease. This may be a consequence of **VEGF** being induced by many of the same inflammatory stimuli (*e.g.*, IL-1, IL-6 and reactive oxygen species) that peak during the acute disease presentation. Geographically, **VEGF** expression was just as strong in the microvasculature of unaffected skin as it was within the purpuric lesions.

One important consequence of **VEGF** expression is the upregulation of different elements of the plasminogen system that modulate the clotting cascade. Plasminogen activation releases fibrin and enables intravascular fibrin deposition. It has been previously appreciated that subsets of HSP patients have coagulation abnormalities, most particularly elevation of von Willebrand factor and the activation and depletion of Factor XIII [28]. A new study of 17 German children with HSP found that 88% had significant elevations in **D-dimer levels**, with half demonstrating values ten times the upper limit or greater [29]. None of the patients had significant alterations in PT, aPTT, or thrombin time. But elevations in **D-dimers**, representing fibrin split products, and increased production of **thrombin metabolites** (seen in 55% of those studied) suggest that a mild form of local coagulopathy may be an important element of the endovascular damage in HSP patients.

Further disruption of microcirculation may be induced by dysregulation of normal homeostatic mechanisms for maintenance of vascular tone. Hyperproduction of nitric oxide (discussed below) could have dramatic effects on local circulation. Another system for regulating vascular tone is that of the **endothelins**, which could be secondarily increased by circulating TNF α in HSP [30]. **Endothelins** are vasoconstrictor peptides synthesized by endothelial cells in response to various stressors. A study of 30 Turkish children in the acute phase of HSP found that endothelin-1 levels were on average three times higher than during the remission phase or when compared with 18 normal controls [31]. Elevated levels did not result in hypertension or increased incidence of renal disease.

As the foot soldiers of the humoral immune response, neutrophils respond to stimulation with activation of an armamentarium capable of killing invading organisms (and bystander cells) in a variety of ways. Factors like **IL8** recruit neutrophils to sites of inflammation, and are produced by local cells in response to many primary stimuli. Many HSP patients have elevated levels of circulating IgA, and tissue deposits of IgA1-containing immune complexes are a hallmark of endothelial damage in HSP. Abnormal glycosylation of IgA1, in particular, appears to play a role in the susceptibility to renal disease [32]. One potential mechanism is the binding of IgA directly to endothelial antigens, with the resultant activation of circulating neutrophils and other innate immune cells that are capable of synthesizing IL8 and other neutrophil chemotactant agents. A new study examined the **specificity of IgA antibodies** in 20 Taiwanese children [33]. They found that TNF α -stimulated human umbilical vein endothelial cells (HUVEC) bound IgA antibodies from 80% of the HSP patients. Using unstimulated HUVEC, the specificity for endothelial cells appears to be specific for IgA (IgM and IgG don't bind appreciably). The phenomenon is not seen in serum from ten JRA control patients and the binding is not diminished by culling out antibodies specific for cardiolipin.

One potent anti-microbial mediator is **nitric oxide**, which in small doses is an essential determinant of vascular smooth muscle tone. At sites of inflammation, however, **nitric oxide** can be produced at hundreds-of-fold greater concentration with potent killing of organisms and significant collateral damage to normal cells. A new study of 25 Turkish HSP patients has shown that serum and urinary nitrate levels are significantly higher during the acute phase of disease than during convalescence or compared with healthy child controls [34]. **Nitric oxide** is a highly reactive molecule that turns into stable nitrate following its interaction with oxygen. They cite a series of similar studies, showing elevated nitrate levels in the circulation and urine of patients with many other forms of vasculitis, including Kawasaki's disease [35].

Lessons and a pathophysiologic model of HSP

“Since the skin is only a limited surface, only a certain amount of rash can occur. But as the amount of the surface area of the skin is exhausted, the internal organs then become affected.”

Collectively, these findings emphasize several important lessons. First, a genetic polymorphism like the multiple tandem repeats in the **IL1RA** gene, which contributes to severity of multiple different autoinflammatory diseases, may begin to explain why multiple different conditions can cluster within a given extended “autoimmune-predisposed” family. Second, these susceptibilities are not simply a global susceptibility to all autoinflammatory conditions; for example, the **IL1RA** alleles have been shown to have no predictive value for either disease severity or susceptibility in adult rheumatoid arthritis [36] or Sjögren’s patients [37]. Finally, one can assume that by working out the details of which conditions are affected by a particular genetic variation, the precise interplay of different genes will begin to come into focus in terms of both who will develop a particular disease and how severely will it be manifested.

In terms of understanding the pathophysiology of HSP, one can begin to imagine circumstances in which selected endothelium is damaged in the context of acute infection by something like Parvovirus B19 [38]. Endothelial cell damage results in increased **endothelin** and von Willebrand factor production, altering local microcirculation. For unclear reasons, specific antibody reactivity with class switching into the IgA locus is induced, possibly related to expression of the **DRB1*01** or other MHC Class II alleles. These antibodies may have specificity for antigens on the surface of activated endothelial cells, including cardiolipin. Formation of Gel & Coombs Type 2 immune complexes with cell-bound antigens results in the arrest of circulating neutrophils, which bear IgA-Fc receptors and which respond by producing **IL8** and other chemoattractants for additional neutrophilic infiltration. Activated neutrophils become factories for the generation of reactive oxygen species and **nitric oxide**, which then cause damage both to the capillaries and to the peri-vascular structures. For unknown reasons, the entire cascade resolves in a matter of days or weeks in most patients. The occurrence of abdominal pain may be related to the HLA genotype (i.e., **B35**) and other factors. Subsequent development of renal impairment occurs in the minority who suffer ongoing consequences of disease, possibly as a result of allelic variation in the **IL8**, **IL1RA** or other genes.

Ultimately, inquiry returns to the question of the precipitating factors for the acute onset of disease. Schönlein had his ideas about this issue:

“The incidence rises and falls, resulting in certain doctors never seeing it. But there were times when there was an epidemic. One remembers 1819 when a lot of doctors were forced to change their opinion. Could it be the composition of the air? Previously doctors thought the water was the culprit, water where a certain grain was roasted. This was because certain regions on the Rhine cultivate hemp, and apparently they wash it and it is aerolized into the air. The way they make their beer with an additive was associated with places where it was common.”

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