Autoimmunity Key: Following Bolton's Cues

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This has been a brilliant composite of talks, representing an almost Gordon conference matrix, and I am honored to be a part of it. I am not personally acquainted with you, Nancy Bolton, but my congratulations for keeping your husband on the straight-and-narrow for these many years. I am sure it has been a challenge, and I praise your stalwart efforts. I do not honestly cast myself in the personal lineage of the Lewis-Bolton-Couser-Salant era, but I have been greatly influenced by the intellectual insights you fathered, and have tried to learn from you and follow your lead. Since this is a Bolton Festschrift, I will concentrate on some of the keys that illuminated my own career path. I would be remiss if I omitted Roger Wiggins, for I tried to follow his advice on becoming a division chief, only to fail dismally at the task. Roger initially told me that I was to go home for lunch with my wife every day. Unfortunately, in that I have absolutely not succeeded.

Figure 1 comes from an era slightly earlier than that of Kline Bolton or Ed Lewis. This is a depiction of the Aristotelian world of medicine – relying on the four basic elements of earth, wind, fire, and water, a variety of good and evil humors, and continuous or discontinuous fevers. There is something very insightful about this cycle today, simply because ancient thinkers never tried to segregate these phenomena; rather, they considered all disease to be in balance. Today, we try to dissect these elements as

Fig. 1. Aristotelian world of medicine – relying on the four basic elements of earth, wind, fire, and water, a variety of good and evil humors, and continuous or discontinuous fevers.

Antineutrophil cytoplasmic autoantibodies (ANCA)

Most crescentic glomerulonephritis and systemic small vessel vasculitis in adults is 'pauci-immune' and associated with ANCA.

ANCA are specific for proteins in the cytoplasmic primary granules of neutrophils and the peroxidase-positive lysosomes of monocytes (e.g. myeloperoxidase and proteinase 3).

Fig. 2. Antineutrophil cytoplasmic antibodies (ANCA).

they pertain to autoimmunity, in more precise terms of genetics, environment, proteins, and cells, and other factors that contribute to answering the question of why one's own immune system attacks itself. This is the question Kline Bolton and his colleagues have been wrestling with for many years.

Why do proteins that are constituents of our normal human composition become the target of autoantigenic expression? Kline Bolton's work on the theory of epitope spreading has shed some light on this, as well as the theory of molecular mimicry, and our own theory of antigenic complementarity. It is most likely that these theories are all partly true and are, in fact, related to one another. Specific disease developing as a consequence of the perturbation of immune regulation can be visualized as a lock mechanism that uses various pins, or tumblers, that will not open without the correct multifaceted key. Kline Bolton has explored some of these facets during his career, and I will use three of his findings as a springboard for my comments.

One of the first modern descriptions of aggressive glomerular injury and its treatment emanated from the University of Virginia. I may have been in middle school at the time of this often-quoted publication of Bolton, Couser and Ben Sturgill describing a group of patients

with crescentic glomerular disease without immune deposits. This manuscript flew in the face of Frank Dixon and his evangelistic approach that every disease had to be of an immune-complex origin. The idea that there was another cause of glomerular disease is really an offspring of this first paper. Kline, Bill, and Ben Sturgill: I have spent my life trying to understand what causes that event – aggressive glomerular disease with no immune deposits.

We now know that 94–95% of cases are pauci-immune crescentic glomerulonephritides associated with antibodies that do not react to the kidney, but react to proteins present on all neutrophils and all monocytes. These anti-neutrophil cytoplasmic antibodies (ANCA) are directed at normal constituents known as 'proteinase 3' and that which makes pus green, 'myeloperoxidase' (MPO; fig. 2). We have now learned that these antibodies are not only serologic markers of the aggressive glomerulonephritis that Kline Bolton and Bill Couser described, but are also part of a group of systemic diseases that involve vessels throughout the human body. We have developed a theory that has now been largely proven in which the leukocytes, or white blood cells, are activated by these autoantibodies and cause vascular damage. Multiple investigators worldwide have demonstrated that neutro-

phils and monocytes are activated when ANCA interact with their autoantigens. These autoantigens, normally well hidden within neutrophils and monocytes, are now expressed. This expression is likely a consequence of cytokine stimulation as well as epigenetic modifications resulting in the loss of silencing of these autoantigen genes. We do know that once the antigens are expressed, antibodies meet their antigens and activate neutrophils and monocytes. Once a neutrophil or monocyte is activated, it interacts with the endothelium, resulting in vascular disruption. This is a wonderful theory, if it could be proven. It proved to be a challenge to our group many years ago. Working with MPO knockout mice, we noted that when the animals were given mouse MPO, an anti-MPO antibody was formed. Lo and behold, when that antibody was passively transferred to naïve animals, vasculitis developed. We have done a number of things to alter the disease. We have revved up the disease by stimulating the animals with cytokines, or downregulated the disease process by eliminating neutrophils. We have now also changed the expression of the disease, not by revving up the antibody or revving up the leukocytes, but by changing the genetics of the animal. In the lung, we have seen capillaritis in some animals, and an entirely different picture resembling human Wegener's granulomatosis is produced when we change the host's genetic background by knocking out an Fc receptor. When we then look at our human population, we have been able to show that humans with lung disease have a functional single nucleotide polymorphism in the same gene as we observed in our mouse model. So, it is not just the antibody and not just the antigen, but it is the host in which these events occur that fashions the phenotype of disease.

There are other phenomena that activate disease as well. Following Kline Bolton's lead, we have been wondering about whether environmental factors stimulate these diseases. I resurrected a paper published by Kline in 1981 on the role of silicon in nephropathy. A cadre of patients is described who had silicosis with a rapidly progressive glomerulonephritis. Two separate manuscripts have explored that same question in our population of patients with small vessel vasculitis. A carefully conducted case-control study by our epidemiologist, Dr. Susan Hogan, showed that there was an association between silica dust and ANCA small-vessel vasculitis. In another case-control study last year, we looked at a much broader population in the southeastern United States. Kline Bolton, you are right again. While there is no risk from low-grade silica exposure, as you increase the exposure over a prolonged period of time, the risk increases. With

a lifetime of low-grade silica exposure, the disease is amplified as well. This is yet another guidepost from the Bolton area that we have followed.

In the past, and even recently, Dr. Bolton and company have been interested in epitope spreading. I would like to share where we have gone with similar kinds of studies. We have been laboring for the last several years on a model of autoantigen complementarity. We believe the initial trigger for this disease is a protein that is complementary to the natural sense protein that makes an antibody response that then triggers an autoimmune process that now reacts to self. We have shown our patients make autoantibodies to both the sense protein and the complementary protein. It is well known that these proteins interact with each other. We have recently used this approach to examine a protein complementary to PR3 in patient sera. Based on the observation that these proteins bind together, we made an antibody in chickens (your favorite animal, Kline) to complementary proteinase 3. We did not use it to create disease, but to make affinity-purified antibodies and extract circulating protein from plasma. The circulating protein turns out to be plasminogen. We then determined that patients are making anti-plasminogen antibodies. We also found that 10% of patients clot, and that these antibodies to plasminogen have a very fine specificity for plasminogen that is homologous with three critical amino acids to complementary PR3. We have used this tool to ferret out other autoantigen approaches as well.

I want to leave you with one last thought, which is an extension of what Bruce Kone talked to you about. We have discussed antibodies at length, and their hosts; but all of us would like to better understand antigens, specifically the antigen I have worked with, called MPO – that which makes pus green. In your studies, did you observe that just one antigen was expressed, or were all sorts of antigenic genes turned on in patients with these diseases? Our microarray data examining gene expression proteins from human leukocytes was less revealing. We looked at all sorts of gene signatures in patients with lupus and ANCA. The lupus gene signature was different from the ANCA gene signature. The gene signatures from the ANCA patients were primarily neutrophil genes. Of course we wanted to know whether the genes for MPO and proteinase 3 were turned on, and almost always together. During disease activity these genes are up, and during disease remission these genes are turned off. In medical school we learned that neutrophils were quiet, or terminally differentiated. Yet in our patients, proteinase 3 and MPO are turned back on in these cells. To touch

Fig. 3. 'Day and Night' by Maurice Escher.

lightly on what Bruce talked about, we have been exploring why normal gene silencing mechanisms are not working. RUNX3 transcription factor is a silencer for both MPO and proteinase 3. A Polycomb complex called PRC2 binds to RUNX3. In patients, RUNX3 is depleted, leading to aberrant silencing. In addition, we have observed increased expression of a histone demethylase in patients with active ANCA disease. I hope that over time, we can understand all of the mechanisms involved, and I would like to be able to turn off the antigen overexpression by getting rid of the demethylase or by making the cells differentiate. What if we could treat these diseases with Rituxan to oust the antibody and another drug to quiet the antigen? This would be a new era that would finally move us away from the treatment that Bolton proposed years ago. Bolton and Couser's use of approximately 30 mg/kg of Solu-Medrol was a huge therapeutic advance. All we have managed to do some 30 years later is to lower the dose. We are using exactly the same approach that Kline Bolton and Bill Couser did so many years ago.

Using art as a model of science, let's look at this picture called 'Day and Night' by Maurice Escher (fig. 3). I think we have been looking at birds, the white birds that have gone from left to right. I think there are a bunch of black birds going the other way. I actually think that it is not just the white birds or the black birds, but actually a combination of antibody and antigen, genetics and environment that, together, inform us about disease in a way that greatly stimulates our own ability to think about autoimmunity in new ways. I hope these new approaches lead to new therapies.

Fig. 4. The 'cause of vasculitis'. Serpent attacking a blood vessel.

Finally, Kline, I am leaving you with this last slide (fig. 4). We found the cause of vasculitis one day by looking at a photomicrograph. Here is a blood vessel with a red blood cell. There is a serpent that is about to chomp on a capillary. I have long wanted to spear this serpent and get rid of those flames emanating from its mouth, just as you have, Kline Bolton, Bill Couser, David Salant, and Ed Lewis. Thank you for providing the original spears aimed at this serpent.