



Review

The Paradigms of Causality and Treatment for Autoimmune Disease

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Abstract. A key concept in medicine is that rational therapy rests on accurate diagnosis; quite simply, therapy that is not tuned to the cause of the disease will not cure the patient. This does not mean that effective treatments cannot emerge from faulty diagnoses. In truth, much of our therapeutic ensemble is composed of drugs developed as a result of chance observation, random screening, intuition or prescientific tradition. Nevertheless, the way to effective therapy is best paved by understanding. Effects are inherent in their causes, so if we want to cure autoimmune diseases using the scientific method, we are obliged to inquire into their causes. By reducing the discordant complexity of the disease to the single cause that underlies it, we can hope to learn the most efficient way to manipulate the disease process. How do we identify a cause when we see one? Quite simply, a single cause is that which is both necessary and sufficient to produce the effect. Here, I explore the general paradigm of autoimmune causality, using multiple sclerosis as a specific example.

Key words: autoimmune disease; therapy; causality; multiple sclerosis; diabetes.

Genes or Environment

Single causes in biology are sought in either of two domains: the genes that program the body and the environment in which the body operates. Health is perceived to be the outcome of a harmonious interaction between one's genome and one's environment, disease the outcome of their discord¹⁰. Thus, we would like to be able to attribute multiple sclerosis (MS) either to a single faulty gene or to a defined pathogenic agent or stress striking the body from the surroundings. Such is the prevailing paradigm.

Therefore, we are frustrated to learn that MS patients, like persons suffering from other autoimmune diseases, cannot be distinguished from those free of MS by any single gene³⁴. Certainly, there is an HLA-asso-

ciated genetic predisposition to MS²⁹, but the great majority of people bearing susceptibility genes for MS, or for any other autoimmune disease, will never contract the disease. HLA genes can only tell us who may be susceptible to developing MS, not who is surely going to get MS. In other words, autoimmune diseases cannot be reduced to a gene in the way that sickle cell anemia can be reduced to a gene for hemoglobin S²¹. Indeed, individuals born with the same genome, i.e. monozygotic twins, have a concordance rate for MS of only about 30%¹². This fact suggests that a factor in the environment must be critical.

However, the development of MS cannot be attributed to a single defined pathogenic agent or environmental circumstance. Viruses, toxins and stresses have all been implicated in certain individuals, but no

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preceding environmental influence can distinguish as a class all of those suffering from MS from those without MS. MS as a category cannot be reduced to an agent or an agency in the way that hepatitis can be reduced to a virus.

Note, parenthetically, that certain autoimmune diseases can be triggered by infection with specific bacteria, such as group A streptococci, which can induce acute rheumatic fever⁶. But even in this example, the majority of those infected with the necessary *Streptococcus* do not develop acute rheumatic fever. Certain viruses, such as HIV or hepatitis viruses, may induce autoimmune pathology with more regularity. An MS virus may yet emerge, but at present, MS cannot be reduced to a single underlying element residing in either the genes or in the environment. Must we conclude that MS has no definable cause? Is MS a matter of bad luck only?

Forbidden Clones

Causality implies order: a predictable effect is inherent in a defined cause¹⁰. Causes, however, can still be random e.g. mutations.

Obviously, it makes sense to try and reduce the development of an autoimmune disease to a renegade clone of autoimmune lymphocytes, the "forbidden clone" of the classical clonal selection paradigm⁷. Clonal selection assumed that any autoimmune lymphocyte would be deleted automatically; hence, autoimmune diseases would have to be accounted for by a somatic mutation giving rise to an autoimmune clone after the critical period of development during which autoimmune lymphocytes are purged from the immune repertoire. This idea is still being taught. The idea is that the immediate cause, both necessary and sufficient for autoimmune disease, is a clone of autoimmune T or B cells. The autoimmune clone might arise in different persons by different accidents, but once it has arisen, the clone is the cause of disease⁴. In other words, the clone is caused by an accident, the disease by the clone. Conceptually, this would put MS in a class with cancer; a transformed cell is the single cause of the tumor, although the transformed cell itself can be generated by accident.

The question then is whether a single population of specifically autoimmune T cells suffices to cause the complex manifestations of an autoimmune disease. This question motivated my colleagues and me to isolate pure cultures of autoreactive T cells *in vitro* and study their effects *in vivo*. We discovered that ex-

perimental autoimmune encephalomyelitis (EAE), considered by many to be a model of MS, could be produced by clones of T cells reactive to myelin basic protein (MBP)⁴. Intravenous administration of about 10^6 activated T cells specific for MBP led to EAE in naive recipient rats. This methodology made it possible to satisfy Koch's postulates and serially transfer EAE from rat to rat by re-isolating the original line of anti-MBP T cells²⁷. Activated anti-MBP T cells demonstrated the ability to migrate to the brain *in vivo*²⁶ and could damage central myelin in tissue culture³⁵. Thus we were able to reduce EAE to specific anti-MBP T cells. Moreover, the cause of EAE could be reduced further to anti-MBP T cell receptors (TCRs), since it is mainly the TCR that distinguishes the different T cell clones^{10, 29}.

Necessary, but Not Sufficient

Soon after reducing EAE to pure cultures of anti-MBP T cells, my colleagues and I went on to transform the forbidden T cell clones into therapeutic reagents by a procedure called T cell vaccination. The idea was to use the autoimmune clones as a vaccine⁴ to activate an anti-clonotypic (anti-idiotypic) response against the renegade cells¹⁹ and so use the immune system itself to rid the body of the cause of EAE. T cell vaccination was found to be effective in EAE and in other experimental T cell mediated autoimmune diseases, and T cell vaccination is now being applied to the treatment of MS patients^{33, 36}. Thus there seems to be confirmatory evidence to support the idea that autoimmune T cells, with their autoimmune TCR, were the cause of autoimmune disease.

However, an idea may still be wrong despite the fact that the idea has produced a correct experiment. The flaw here is that autoimmune T cell clones may be necessary for disease, but they are not sufficient for disease. Rats which had recovered from EAE and were resistant to future bouts of the disease were found to harbor clones of potentially virulent anti-MBP T cells^{3, 8, 24}. Thus, EAE could not be reduced to the presence or absence of anti-MBP T cell clones; the clones were surely necessary but not sufficient for EAE. Thus, single aberrant clones of autoimmune lymphocytes are not the cause of autoimmune disease in the way that single aberrant clones of transformed cells are the cause of cancer. The insufficiency of anti-MBP T cells as the cause for EAE was reinforced by the findings that healthy rats are populated with anti-MBP T cells and, despite the presence of the clones, the rats will never

develop EAE unless they are actively immunized to MBP or receive activated anti-MBP T cells^{3, 24}. Anti-MBP T cells are also easily recovered from healthy people, and not only from persons with MS³¹. In fact, anti-MBP T cells probably help maintain the central nervous system (CNS) in the face of trauma; we recently found that such cells mediate neuroprotection²³. We did not know whether anti-MBP T cells are necessary for MS; the presence of such T cells is certainly not sufficient to cause MS. We must return to the question we raised above: can MS (or EAE) or other autoimmune diseases be reduced to a causal element that is both necessary and sufficient?

Structures

It is obvious that many structural elements are necessary to generate MS or EAE: MHC susceptibility genes, T cells with specific TCR types, target antigens and peptides presented in the nervous system, accessory molecules involved in T cell recognition and cell traffic, enzymes to penetrate tissues, inflammatory cytokines and inflammatory leukocytes, and so forth. But all of these elements are present in healthy persons. None of the elements is abnormal. What can there be wrong with healthy elements that allows them to cause a disease?

The search for the cause of MS has been frustrated because we tend to think of causality, in essence, as structural. We are satisfied intellectually when complex actions can be attributed to simple underlying structures¹¹. After all, structure determines function¹⁰. Water, for example, functions the way it does in biology as well as in chemistry because of the fundamental dipolar structure of the water molecule. An antibody recognizes its specific antigen by virtue of the structure of the antibody's combining site¹⁰. Many scientific explanations are founded, in principle, on the discovery of the basic structures responsible for the observed functions. In biology the reduction of function to defined structures is especially valuable because such discoveries, as stated at the outset, lead to rational therapy. All the complex expressions of sickle cell anemia can be understood to result from the structure of the gene for hemoglobin S. The diagnosis of a sick gene prescribes an ideal treatment, that of gene therapy, irrespective of whether gene therapy is presently feasible. Hepatitis is caused by the structure of a particular virus; thus the ideal therapy is to get rid of the virus (various options are conceivable). The principle of reduction to structure motivates rational therapeutic innovations in immunology, too.

The reduction of an autoimmune disease to the structure of the TCR is a diagnosis that entails particular therapies. If renegade clones are the culprits, then rational therapy would be to kill or inactivate the TCR-bearing T cells, or to block the recognition by the TCR of the MHC-target peptide complex. Interference with antigen recognition has been the aim of blocking peptides, antibodies to the MHC, to the MHC-peptide complex, to the TCR, or to other ancillary ligands involved in T cell recognition, such as the CD4 molecule². Is such structural reduction going to work in therapy for MS? Can we cure the disease by getting rid of some discrete structure?

Interactions

Despite the best efforts, I doubt whether we shall be able to cure MS by blocking or getting rid of some specific structure, either a cell or a molecule. This is because the disease MS is caused not by a T cell clone, a structure, but by an **interaction**, the way the T cell clone interacts with other lymphocytes, with the blood vessels and the CNS. The disease is caused by the fact that the clones are activated in the CNS in a way that leads them to secrete pro-inflammatory cytokines, IFN- γ , TNF- α and others, which generate damaging inflammation leading to demyelination and the signs and symptoms of MS^{30, 32}. Thus, MS and EAE cannot be reduced to discrete structures, but to interactions between structures, undesirable interactions between otherwise healthy structures¹⁰.

For clarity, reduction to interactions might need a terminology to distinguish this type of causality from reduction to structure. The term **interactional causality** might be suitable because the effect emerges from a context of interactions rather than from a specific underlying structure.

Interactional Therapy

If autoimmune diseases are caused by interactions and not by underlying structures, should we change the way we do basic and clinical research in the autoimmune field? The answer depends on how one looks at the question. As in the past, we still must persist in the classical work of structural, reductive science and identify the various cells and molecules that are necessary to produce MS¹⁰. But we would probably do better to think about the disease process more comprehensively and consider the context of interactions of these cells

and molecules. In other words, autoimmune diseases will have to be reduced to their requisite structural ingredients: genes, gene products and other molecules and cells. But we probably would not be able to cure the disease rationally (we might stumble onto the cure by accident) unless we consider the critical interactions. The cure will come not by **inactivating** any of the key structural elements, but rather by using key structural elements to **activate** new or corrective interactions⁹.

A concrete example of therapeutic activation has emerged from the work of my colleagues and me on the spontaneous autoimmune diabetes developing in non-obese diabetic NOD strain mice. We began by identifying some key structural elements that were not previously thought to be involved in the disease. We discovered that T cells reactive to a stress protein, the 60 kDa heat shock protein (hsp60) of the mouse, could mediate disease in healthy recipient mice^{16, 18}. The anti-hsp60 T cells were not sufficient to cause diabetes because healthy individuals also harbor such T cells²⁵. Nevertheless, the pathogenic T cells served as a probe to help us identify a target peptide in the hsp60 molecule^{13, 19}. We then went on to test the interactions of the hsp60 peptide and how it could influence the autoimmune diabetic process. The peptide was found to activate opposing effects depending on the context of its administration. Conjugated to a foreign carrier molecule, the self-peptide could activate an autoimmune effector response leading to insulinitis and hyperglycemia¹⁵. In contrast, the same peptide unconjugated to another immunogen could induce the arrest of the autoimmune process, even when it was far advanced^{13, 14}. It is now clear that hsp60 – peptide therapy activates a burst of peptide-specific Th2-type T cells (producing anti-inflammatory cytokines IL-4, IL-10 and probably TGF- β) that abort the inflammatory process produced by the Th1-type T cells (producing IFN- γ and IL-2) that actually penetrate the islets and inactivate the insulin-producing β cells¹⁷. The Th2-type of interaction resets the cytokine profile and down-regulates the Th1-type responses to other β -cell antigens such as insulin and glutamic acid decarboxylase. Interestingly, therapeutic vaccination with the hsp60 target peptide led to the activation of anti-idiotypic T cells that recognized the T cell receptor of the pathogenic autoimmune T cells²⁰. In other words, the Th2 cytokine shift was accompanied by anti-idiotypic regulation; both forms of immune regulation would seem to be linked functionally. Most importantly, these modifications of the autoimmune interaction are expressed clinically as to cure advanced disease in the majority of the mice¹⁴. Therapeutic pep-

ptide vaccination is now being tested clinically in humans suffering from recent onset type 1 diabetes mellitus.

In this example, we see that the cells and molecules that structure the immune system can be left intact and that only their interactions need be influenced by a key control element to shift the system away from a pathogenic interaction¹⁰.

Therapy for MS

I would now like to discuss the prospects for specific immune modulation of a disease such as MS. If we perceive MS to be caused by pathogenic interactions, then it makes sense to try and activate the immune system using information that will induce corrective or new interactions within the autoimmune response that causes MS⁹. In other words, MS might be susceptible not to immune suppression, but to immune activation. T cell-vaccination⁵ is one way of activating immune regulation^{19, 22} and clinical trials are underway^{33, 36}. Of course, we can also consider exploiting the Th1-Th2 paradigm; epitopes of MBP or other relevant antigens might be administered in a cytokine or adjuvant context that activates a switch from a pro-inflammatory outcome to an anti-inflammatory outcome of the autoimmune response^{1, 17}. The success of the activation approach, using either T cell or peptide vaccinations, would depend on two conditions: the activation would have to be given early in the course of disease, or during the early remitting phase. Once MS progresses to a chronic unremitting phase, the disease process has become robustly entrenched and may no longer be susceptible to interactive manipulation. Indeed, the capacity of MS to remit spontaneously can be taken as evidence that the autoimmune response is still naturally susceptible to shifting interactions.

A second condition for activation therapy is that we discover surrogate markers for the pathogenic and for the therapeutic types of interaction. We need surrogate markers because we cannot wait for years to pass to see the outcome of therapy. We must know if we are on the right track. Has the patient responded as we desire? Is the dose of peptide sufficient for the particular patient? Is a booster activation needed? If, for example, secretion of IFN- γ by anti-MBP T cells were a sign of a pathogenic interaction and secretion of IL-4, IL-10 or TGF- β were signs of a healthy interaction, then we could use such markers to tailor activation therapy to the patient's needs. Time will tell.

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