

Oxidative Stress, Altered-Self and Autoimmunity

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Abstract: It is well known that oxidative stress can induce production of free radicals that can modify proteins. The hypothesis presented here is that the target antigens in autoimmune diseases, such as type 1 diabetes mellitus, are not normal self proteins but rather self proteins that have been altered by free radicals, produced as a result of oxidative stress in the target cells. The observation that disease often occurs only in a single tissue, even when other tissues contain the same antigen, could be explained if both the presence of the antigen and severe oxidative stress is required, before tissue destruction occurs. Furthermore, the cyclic nature of tissue destruction and problems with detecting robust high affinity autoreactivity to self antigens may be easier to understand if we presume that pathological autoreactivity is targeting redox-modified self antigens, while assays to unmodified self only measure its cross-reactivity to normal self antigens.

BACKGROUND

Autoimmune diseases such as type 1 diabetes mellitus (DM1) are believed to result from the failure of immunological tolerance to protein self antigens. It has been proposed that alterations in self antigens could initiate the process of autoimmunity [1, 2]. According to this hypothesis one does not need to assume that autoimmune diseases are initiated exclusively by imperfections in the ability of the immune system to tolerize itself to normal cellular components (self antigens). Instead, the self antigens change to a previously unseen form and, therefore, become a natural target of the immune system. The immune response to the altered self antigen then progresses and helps develop immunity to unaltered self antigens as well.

If the mitochondria are dysfunctional or cells are under stress, such as during high metabolic demand, viral infections, or exposure to certain cytokines/toxins, cells may produce a sufficient amount of radical oxygen or radical nitrogen species (ROS/RNS) to overwhelm the antioxidant systems that normally neutralize these free radicals [3]. These ROS/RNS are very short-lived because they react with and modify, lipids and proteins in the cell [4]. The field of redox proteomics has been progressing rapidly in the past decade and has developed new methods that allow investigators to study a large number of specific modifications of proteins induced by free radicals [5-7]. The reaction of ROS/RNS with proteins can create stable end products such as 3-chlorotyrosine and 3-nitrotyrosine that may not only block natural modifications of the tyrosine by e.g., phosphorylation, but also change the antigenic profile of the protein. The reaction of hydrogen peroxide with the chloride anion can create hypochlorous acid, which deaminates serine and con-

verts it into glycoaldehyde. These aldehydes not only change the antigenic profile of a protein, but actually enhance the antigenicity of proteins, turning non-immunogenic malaria antigens into effective immunogens [8].

A number of oxidative protein modification have been described in autoimmune diseases [9]. In systemic lupus erythematosus the 60kD Ro ribonucleoprotein is one of several autoantigenic targets. Autoimmunity was initiated at an accelerated pace in animals immunized with oxidatively modified 60kD Ro protein [10]. Oxidative modifications produced high molecular weight complexes of glutamic acid decarboxylase (GAD) and sera from type 1 diabetic patients bound these complexes much more strongly than the monomer GAD autoantigen [11]. It has been shown that several of the autoantigens targeted in diffuse scleroderma are uniquely susceptible to cleavage by ROS [12]. Oxidation of beta-2-glycoprotein, a target of antiphospholipid antibodies with hydrogen peroxide rendered this protein able to activate immature monocyte-derived dendritic cells [13].

It has been shown that the insulin-producing beta cells in the islet of Langerhans are particularly vulnerable to damage by free radicals [14]. Furthermore, beta cells appear to express relatively low levels of some antioxidants and are slow to increase their expression in response to oxidative stress [15]. That would leave the beta cells particularly susceptible to modifications on their proteins when ROS/RNS production is increased. Metabolic, infectious, or other types of stress on the cells could, therefore, result in substantial levels of redox modification on proteins in the cells (Fig. 1).

HYPOTHESIS

The hypothesis presented here is that: the targets of pathologic immune responses in autoimmune diseases such as DM1 are not normal self proteins but rather self proteins that have been altered by ROS/RNS, produced as a result of oxidative stress in the target cells. The principal challenge to

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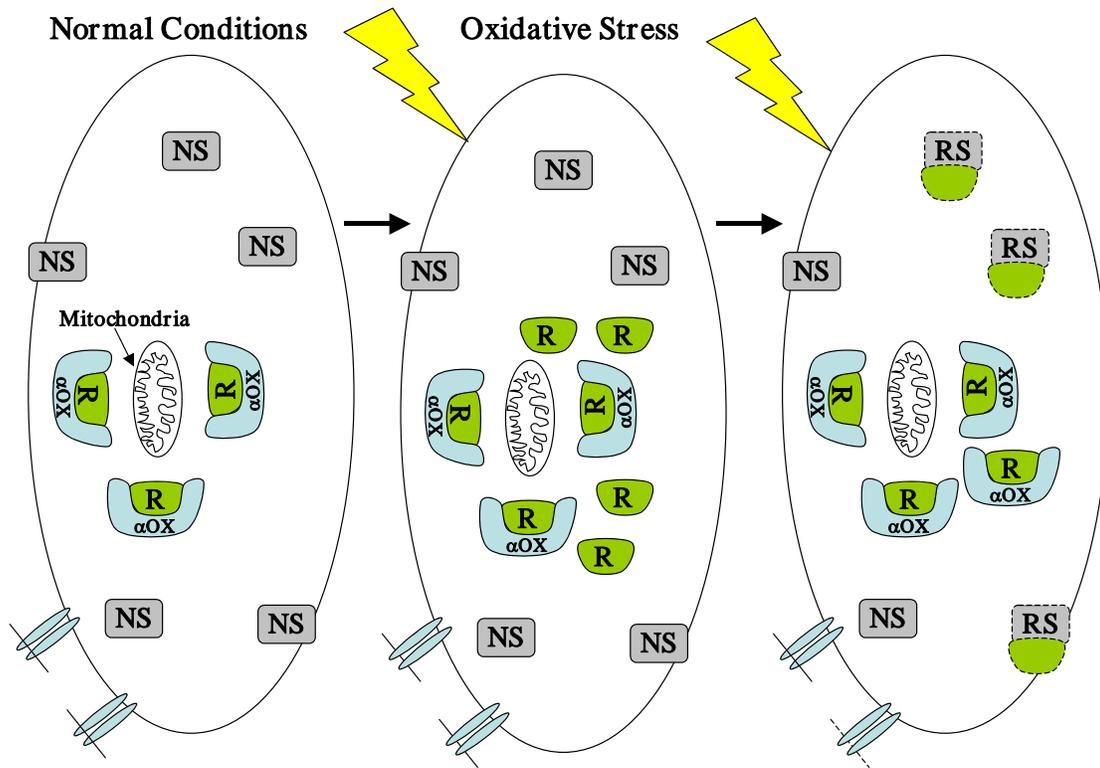


Fig. (1). Alteration of self proteins by oxidative stress. Mitochondria inside a cell produces free radicals (R) that are neutralized by antioxidant defenses (α OX) such that normal self antigens (NS) remain protected from damage by free radicals. Under conditions of increased free radical production (oxidative stress) the antioxidant defenses may not be able to neutralize all the free radicals produced. As a result, some of the normal self antigens are targeted by free radicals and become radical modified self antigens (RS) structures that the immune system may not have been tolerized to and, therefore, will develop responses against. The altered self recognized by the immune system could either be expressed directly on the surface of the cell or as peptide fragments of internal antigens presented by MHC class I molecules on the surface of the cell.

this hypothesis is to explain the fact that assays used to detect autoimmune responses often use antigens isolated from normal tissues or normal proteins/peptides. However, a considerable amount of stress is induced on tissues such as islets of Langerhans during their isolation. Furthermore, synthetic peptides and purified/recombinant proteins often have prolonged exposure to the oxygen in normal air and could undergo oxidizing modifications as a result of that exposure. Therefore, a small fraction of the purified normal self antigens could be redox-modified. That small fraction could provide a signal for “autoreactivity” in assays of reactivity to (presumed) normal-self. Even if the autoantigen preparations used are completely devoid of redox modifications, an immune response to modified self could have enough cross reactivity to the corresponding normal self epitopes that it would give a signal in some of the autoreactivity assays, even if that reactivity is not strong enough to be of pathological relevance. This would be particularly likely to occur with detection of autoantibodies, because the requirement is just for sufficient binding affinity to not be “washed off” by a particular buffer.

While this hypothesis initially seems fairly easy to test, it requires careful consideration of experimental designs and limitations. It should not be presumed that we are dealing with an all-or-none phenomenon, but rather that there is some cross reactivity between the normal self antigen and the redox-modified self antigen. The immune response to

normal self is presumed to be sufficiently lower than that to redox-modified self to render the former of little or no pathological consequence. Yet any preparation of normal self antigen and redox-modified self antigen is unlikely to be 100% pure and will most likely contain a mixture of both. Fortunately there are now fairly reliable methods to quantitate the amount of most redox modifications [16] and self antigens, thus for each specific preparation it should be possible to define its relative content of both the modified and unmodified forms of the autoantigen. Combined with traditional titration of binding affinities it should be possible to show if there is indeed a stronger “autoreactivity” to self antigens and self epitopes if they have undergone redox modifications. Another complication is the large number of different modifications that can be induced by ROS/RNS. Because each modification will change the molecular bulk of the antigen in different ways, each may have to be studied separately.

DISCUSSION

One implication of this hypothesis is that immunological tolerance to true self antigens is never broken in autoimmune diseases such as DM1, and that idea may appear somewhat provocative. However, this assumption may help explain certain clinical phenomena in DM1 that have been somewhat difficult to understand otherwise. It is well known that after initiation of treatment with insulin in newly diagnosed DM1

patients their production of C-peptide and needs for insulin may decrease, even to the point of making some patients insulin-independent for up to a year after initial diagnosis (the honeymoon phenomenon). This increase in the patient's own insulin production suggests that the immune destruction of beta cells diminishes after initiation of insulin therapy. Such temporary reductions in immune responses, even though the antigen is still present, have not been reported for other types of immune responses. Indeed, it has generally been found that if a virus or bacteria is re-introduced, the secondary immune response occurs both faster and with greater magnitude than the original response. The honeymoon phenomenon may be easier to understand in the context of the hypothesis presented here. In the period before diagnosis of DM1, beta cells are under severe metabolic stress because a dwindling number of these cells tries to keep up with a constant demand for insulin. However, after the initiation of insulin injections in the patient, this metabolic demand, on remaining or newly generated beta cells, would be drastically reduced, as would their ROS/RNS production. As a result, the remaining beta cells would have a drastic reduction in the amount of redox-modified self antigens they express, and become much less of a target for an immune response to redox-modified self antigens. Yet, the inevitable imperfections in insulin therapy would eventually expose the remaining islets to high glucose and the accompanying oxidative stress. Therefore, the honeymoon phenomenon may be easier to explain in the context of immunity to redox-modified altered self than in the context of an immune response to true self antigens. It should also be noted that a substantial number of patients diagnosed with type 2 diabetes have been shown to have autoantibodies in their plasma [17, 18]. Although the original pathology in type 2 diabetes is not related to autoimmune destruction of beta cells, it is certainly possible that oxidative stress and metabolic demand on beta cells in these patients could provoke development of pathogenic beta cell immunity to redox modified autoantigens, as a later complication.

Although many lines of evidence suggest a central role for T lymphocytes in DM1, it has been difficult to define beta cell antigens and epitopes with strong signals of T-cell autoreactivity in blood from DM1 patients. Stimulation indexes and other measures of T-cell autoreactivity are often considerably smaller than what is seen in measurements of other forms of T-cell reactivity [19-21]. That problem may be easier to understand in the context of this hypothesis. The T-cell receptor is presented with a small peptide fragment of its autoantigen [22], and the presence or absence of an oxidative modification could represent a substantial difference in the molecular "bulk" of such a small molecule. As a result, the binding of a T-cell receptor that is specific to a redox-modified peptide would likely be considerably weaker if it was presented with the non-modified form of the same peptide.

The hypothesis that altered self is the target in organ-specific autoimmune diseases, such as DM1, could also help explain why the autoimmune responses are organ-specific and do not attack all tissues that express the target antigens. A tissue would only be targeted if it both expresses the autoantigens and also is undergoing a level of oxidative stress that overwhelms its antioxidant defences. Furthermore, depending on the reason for oxidative stress it may not be

constantly present in a given tissue, leading to periods of more or less activity of the disease.

Many years ago it was reported that when serum from newly diagnosed DM1 children were used to immunoprecipitate the 64kD autoantigen (later identified as GAD) from mouse islet of Langerhans, the immunoprecipitated band was more intense if the islets came from mice infected with an islet tropic strain of Coxsackie virus B4 [23]. At that time it was concluded that expression of the 64kD autoantigen in the islets had been increased by the virus infection. However, in light of the above hypothesis an equally possible explanation would be that the infection could have increased oxidative stress and redox-modified GAD in the islets that could be immunoprecipitated by the DM1 patient serum.

From a treatment point of view this hypothesis would suggest that, in addition to therapies targeting the immune system, we should also consider developing therapies that decrease oxidative stress in the target tissue. Such therapy could be used both for prevention and early treatment of the disease. Furthermore, tolerance inducing therapy should be using redox-modified autoantigens rather than normal autoantigens to be effective.

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ABBREVIATIONS

DM1	=	Type 1 Diabetes Mellitus
ROS	=	Radical Oxygen Species
RNS	=	Radical Nitrogen Species
GAD	=	Glutamic Acid Decarboxylase

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