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Review

The Role of Pathogenic Autoantibodies in Autoimmunity

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Abstract: The serological presence of autoantibodies is diagnostic of autoimmunity, and these autoantibodies may be present for many years before the presentation of autoimmune disease (AID). Although a pathogenic role has been demonstrated for various autoantibodies reactive with cell surface and extracellular autoantigens, studies using monoclonal antibodies (mAb) show not all antibodies in the polyclonal response are pathogenic. Differences depend on Fab-mediated diversity in epitope specificity, Fc-mediated effects based on immunoglobulin (Ig) class and subclass, activation of complement, and the milieu in which the reaction occurs. These autoantibodies often occur in organ-specific AID and this review illustrates their pathogenic and highly specific effects. The role of autoantibodies associated with intracellular antigens is less clear. *In vitro* they may inhibit or adversely affect well-defined intracellular biochemical pathways, yet, *in vivo* they are separated from their autoantigens by multiple cellular barriers. Recent evidence that Ig can traverse cell membranes, interact with intracellular proteins, and induce apoptosis has provided new evidence for a pathogenic role for such autoantibodies. An understanding of how autoantibodies behave in the polyclonal response and their role in pathogenesis of AID may help identify populations of culprit B-cells and selection of treatments that suppress or eliminate them.

Keywords: autoimmune disease; monoclonal antibody; immunoglobulin; epitopes; Fc receptors; intracellular antigens; autoantibodies

1. Introduction

The first description of a pathogenic autoantibody by Donath and Landsteiner in 1904 [1] was a landmark discovery in the history of clinical medicine. For the second half of the 19th century clinicians had grappled with the definition of a condition that was characterized by "paroxysms" induced by cold and followed by hemoglobinuria. Typically, but not exclusively, these episodes occurred in subjects with syphilis. The "paroxysm" preceding hemoglobinuria varied in presentation ranging from absence of symptoms to severe backache, abdominal cramps, headache, pyrexia and, in some cases, acute anemia despite the absence of erythrocytes in the hemoglobin-stained urine. The episode was given the descriptive term paroxysmal cold hemoglobinuria (PCH) but the etiology remained unknown until the elegant laboratory studies of Landsteiner demonstrated the sequence of immunological events that occurred in the blood of three subjects with PCH under the care of Donath.

Landsteiner showed that the erythrocytes of these subjects when cooled in an iced water-bath, became "sensitized" by a factor in their serum (or plasma). Then, when the temperature was raised to body temperature and heat-labile factors were present in their serum, their erythrocytes underwent lysis liberating hemoglobin. The autohemolysin had all the hallmarks of a pathogenic autoantibody but, in 1904, little was known about the nature of antibodies, let alone about heat-labile factors in serum.

Autoimmunity was first identified as a cause of disease based on the recognition of self-reactive autoantibodies in the sera of patients. In these early studies, the autoantigens were not well defined and antibodies were considered in terms of reactivity with whole cells, cell extracts or relatively impure antigen, and disease was attributed to these autoantibodies. The first detailed descriptions of experimental induction of autoantibodies, presumed to be pathogenic, were those of Rose and Witebsky [2] who produced autoimmune thyroiditis in rabbits with extracts of thyroid glands. Subsequently similar autoantibodies were demonstrated in the comparable human disease, Hashimoto's thyroiditis [3].

During the latter half of the 20th century many diseases were identified as autoimmune, poorly understood groups containing such diseases were reclassified and immunosuppression became a standard method of treating the chronic inflammation of autoimmunity. Hand in hand with these advances was the discovery that antibodies, although an integral part of the immune system, were not the only means of immunological defense and the immune system was, in fact, a vast and complex network of cells, cytokines and regulatory mechanisms adapted to maintain an optimal state of health in a hostile environment. Although autoantibodies have revolutionized the diagnosis of autoimmune diseases their role in pathogenesis has not always been obvious. Many of apparently unrelated specificities cluster within particular diseases and whilst their association with a disease is undeniable their role in pathogenesis is unclear.

As a template for nominating pathogenic autoantibodies, in this review we sought irrefutable evidence for their pathogenicity in a range of autoimmune diseases, examined the mode of action of antibodies within the polyclonal response, and then scrutinized the role of a number of pathogenic autoantibodies in diseases representative of the entire spectrum of autoimmune diseases.

2. Evidence for Pathogenicity

Various techniques have been used to examine the pathogenicity of autoantibodies (Table 1). The most convincing evidence comes from the development of disease as a result of the direct transfer of autoantibodies. In a minority of autoimmune diseases this occurs by transplacental transfer of immunoglobulin G (IgG) from an affected mother. The effect of the maternal pathogenic autoantibody on the fetus can range from death *in utero* to transient disease, with recovery as the parental IgG declines in the neonate.

Table 1. Examples of pathogenic autoantibodies.

Autoantigen	Disease	Induction of Disease by Autoantibody				*In Vitro* Effects	B Cell Depletion (Rituximab)	Plasmapheresis/ Plasma Exchange/IvIg
		Transplacental Transfer	Human to Human	Human to Animal	Animal to Animal			
Erythrocytes	Autoimmune hemolytic anemia	yes [4]			yes [5]			
Platelets	Autoimmune thrombocytopenic purpura	yes [6]	yes [7]				yes [8]	
Desmogleins Dsg3, Dsg1	Pemphigus vulgaris Pemphigus foliaceus	yes [9–13]		yes [14,15]	yes [16,17]	yes [18]	yes [19]	IvIg [20]
BP180/ collagen XVII	Bullous pemphigoid Herpes gestationis	yes [21,22]		no [23] antigen differs	yes [24–27]	yes [28] Requires neutrophils		yes [29] IvIg [20]
Collagen VII	Epidermolysis bullosa acquisita			yes [30] limited cross-reactivity	yes [31]	yes [32] Requires neutrophils		yes [33] IvIg [20]
TSH receptor-stimulatory-blocking	Graves' disease	yes [34] yes [35,36]	yes [37]					
Intrinsic factor H^+/K^+ ATPase	Autoimmune gastritis			yes [38]		yes [39]		
M3 muscarinic receptor	Sjögren's syndrome			yes [40,41]				IvIg [42,43]
Acetylcholine receptors	Myasthenia gravis	yes [44,45]		yes [46,47]	yes [48]		yes [49]	yes [50,51]
Presynaptic voltage-gated Ca^{++} channels	Lambert Eaton Syndrome	yes [52]		yes [53–55]				yes [51]
Collagen II	Rheumatoid arthritis			yes [56]	yes [57]	yes [58–62]	yes [63]	yes [64] Limited efficacy

Experimentally, pathogenicity has been tested by examining the effects of short-term infusion of autoantibodies, either to human subjects [7,37,65] or to animals, usually mice (Table 1). In these cross-species transfers, the amount of antibody and duration of infusion has been limited, and clear evidence for pathogenic effects has been confined to transfer of autoantibodies reactive with cell-surface antigens, including cell adhesion molecules [14], receptors and ion channels [46,53,66], or components of the extracellular matrix, particularly collagens [31,57,67].

Studies of direct transfer of antibodies have been supplemented by the development of animal models of diseases. For a number of autoimmune diseases, there are spontaneously occurring models, but although these provide essential insights into the genetics and processes pertaining to the development of autoimmunity, their complexity including T-cell interactions limit their utility for examining the pathogenicity of autoantibodies. However, useful models that are T-cell independent have been developed either by transfer of monoclonal antibodies from an immunized animal, as has been done in collagen antibody induced arthritis (CAIA) [57], or, where human autoantibodies are unreactive with the mouse equivalent, by transfusing mice with anti-mouse antibodies derived by immunizing another species with mouse antigen [27,31]. In these cross-species transfers, the amount of antibody and duration has been limited, and gross pathogenic effects are often difficult to observe, but a mouse model of pemphigus vulgaris has been developed in which there is continuing exposure to autoantibodies without concomitant T cell responses by injecting the mouse desmoglein Dsg3 into $Dsg3^{-/-}$ mice, then transferring splenocytes from those mice into $Rag\text{-}1^{-/-}$ immunodeficient mice that then continue to produce autoantibodies [16].

Evidence for pathogenicity has also come from clinical improvement after removal of autoantibodies by plasma exchange or plasmapheresis, or from B cell depletion with drugs such as Rituximab (anti-CD20), or with intravenous immunoglobulin (IVIG), for which the mode of action is still unclear, but may involve replacement of anti-idiotype antibodies.

3. Mode of Action

In the normal immune system, the production of antibodies is triggered by foreign proteins, microorganisms or toxins, and antibodies are responsible for their elimination, both by direct inactivation, and by activating secondary effector cells. Antibody responses can be categorised in terms of a primary reaction involving the antigen-binding site (paratope) of the Fab region of the antibody with the specific epitope on the cognate antigen combined with secondary effector functions, both complement-mediated and cellular, through interactions of the constant regions of the heavy chain, particularly the Fc. Similar events occur also in autoimmune responses.

3.1. Fab-Mediated Effects

Binding of $F(ab)_2$, monomeric Fab, or single chain variable domain fragments (ScV) is readily demonstrable for most antigens *in vitro*, but evidence of direct pathogenicity of Fab has been demonstrated for relatively few autoantigens, as such tests require not only a source of relatively pure antibody to prepare the Fab, but also an appropriate functional assay to test the effect of binding. The antigen must be accessible to Fab binding, and the binding must alter some biological function of the exposed antigen, whether by competing with interactions with other proteins, or by blocking a cellular function. For example, to show the pathogenic effects of $F(ab)_2$, Fab, or ScV from antibodies to desmoglein in pemphigus vulgaris, pathogenicity was assessed by the development of skin blisters following injection into newborn mice [68–71]. In another functional assay, electron microscopy was required to show that $F(ab)_2$ from a monoclonal antibody to collagen II inhibited normal collagen fibril formation in chondrocyte cultures [59].

F(ab)-mediated functional effects have been shown for autoantibodies to a range of surface receptors and ion channels. For some, such as autoantibodies to the M3 muscarinic receptor where monomeric F(ab) is inhibitory, these may block cell function directly, but for others, where functional effects are seen with $F(ab)_2$, but not Fab or ScV, changes have been related to loss of surface receptors following cross-linking and internalization of surface receptors with the divalent $F(ab)_2$ [72–75].

Less directly, F(ab)-mediated effects could be modified by the somatic hypermutation that occurs during affinity maturation. The paratope is formed from three hypervariable complementarity determining regions (CDRs) that are separated by four framework regions which have more stable amino acid sequences, and these hypervariable regions undergo considerable somatic mutagenesis during affinity maturation [76,77]. As not all the amino acids in the CDRs for any particular antibody are involved in antigen binding, somatic hypermutation can occur not only in residues within the paratope, but also in adjacent areas not originally involved in antibody binding. Such mutations could not only change the affinity of binding, but also modify the epitope recognised, or change the properties of the antibody, and may affect interactions with anti-idiotypic antibodies [78].

3.2. Fc-Mediated Effects

The structure of the paratope of the Fab region defines the specificity and affinity with which antibodies bind with their cognate antigen. Factors that amplify the effect do not depend on the antigenic specificity, but on the properties of the immunoglobulin class or subclass. These secondary effects depend on the structure of the Fc-region, which defines the Ig isotype or subclass of the antibody, and plays a critical role in amplifying the effect of an antigen-antibody interaction by complement activation, or binding to Fc-receptor (FcR)-bearing effector cells, such as monocytes, macrophages, dendritic cells, neutrophils, mast cells, and natural killer cells. Four Fcγ receptors have been described that bind IgG subclasses with differing affinities, and have different functions (Table 2), and Fc receptors have been described that bind to each of the immunoglobulin classes. The FcαR1 (CD 89) binds IgA, Fcα/μ binds IgM and also IgA but with lower affinity, and two Fcε receptors bind IgE.

Table 2. Properties of human IgG subclasses.

			IgG1	**IgG2**	**IgG3**	**IgG4**
Proportion of total IgG			66%	23%	7%	4%
Crosses placenta [79]			++	±	+	+
Complement activation			++	+	+++	–
C1q binding and activation [80]			+	–	+++	–
Complement–mediated hemolysis [80]			+++++	–	++	–
C4 activation [80]			+++++	–	++	–
Binding to Fcγ Receptors [81,82]		**Major Function**	**Binding Affinities (M^{-1})**			
Fcγ R1 (CD64) "high affinity receptor" monocytes, macrophages, neutrophils, dendritic cells	Fcγ R1	Activation	**6×10^7**	No binding	**6×10^7**	**3×10^7**
Fcγ RII (CD32) "low affinity receptor" monocytes, macrophages, neutrophils, eosinophils, platelets, B cells, dendritic cells, endothelial cells	Fcγ RIIA	Activation	5×10^6	4×10^5	9×10^5	2×10^5
	Fcγ RIIB	Inhibition	1×10^5	2×10^4	2×10^5	2×10^5
	Fcγ RIIC	Activation	1×10^5	2×10^4	2×10^5	2×10^5
Fcγ RIIIA (CD16) "low affinity receptor" neutrophils, eosinophils, macrophages, NK cells, subsets of T cells	Fcγ RIIIA	Activation	2×10^5	7×10^4	1×10^7	2×10^5

Bold: High affinity.

In the adaptive immune response, complement activation is initiated via the classical pathway by C1q binding with the Fc region of antibodies in immune complexes [83]. Both IgM and IgG antibodies activate complement, IgM more efficiently, and there are differences in the activity of the IgG subclasses, both in initial binding with C1q, but also in effects in activating later steps in the complement pathway (Table 2). Also, binding of the Fc region of the Ig to the extracellular parts of the α chains of the FcR leads to cross-linking and activation of tyrosine kinases interacting with intracellular FcR domains, stimulating phagocytosis, degranulation and release of cytokines and inflammatory mediators, and antibody-dependent cellular cytotoxicity. Different FcRs are expressed by different cell types, with different biological activities that are modulated by aggregation by multivalent antigen-antibody complexes. Most cells express several FcRs, and different FcRs can generate different signals to a single cell. In addition, different immunoglobulin classes, and particularly IgG subclasses, bind to different FcRs (Table 2). Together, these interactions allow considerable diversity of responses to any antigen. Both stimulatory and inhibitory FcγR receptors can occur and there is a complex interaction between the effects of FcγR and complement [84,85]. As different Ig subclasses differ in their capacity to activate complement, and bind with differing affinity to different FcγRs, autoantibodies can differ not only in their antigenic specificity, but also in secondary pathogenic effects related to their immunoglobulin subclass.

The importance of Ig classes and subclasses in determining the pathogenicity of autoantibodies has been shown in studies of murine autoimmune hemolytic anemia, induced by monoclonal antibodies (mAbs) to mouse red blood cells from hybridomas from adult anemic NZB mice. The mAbs were pathogenic in BALB/c mice, and caused marked anemia with a single injection. The anemia resulted from sequestration of agglutinated cells in spleens and liver, with erythrophagocytosis by Kuppfer cells in the liver, and not from complement-dependent hemolysis [5]. Four IgG class-switch variants of two of these mAb were derived that retained their epitope specificity. Their use to induce haemolytic anemia by injection into BALB/c mice demonstrated clearly that there were major differences in pathogenicity according to the IgG subclass of the antibody. The extent of anemia that developed following a single injection did not depend on the epitopes recognised, but correlated with Fc-receptor dependent phagocytosis and complement activation [86–89].

Although most Fc-receptors are located on immune effector cells, Fc receptors can occur on other cells. For example, various Fcγ receptors occur in the brain, on microglia, astrocytes, oligodendrocytes and neurons, where they may facilitate immunoglobulin uptake and transport to the cell body [90,91]. Other specialised Fc-receptors transport immunoglobulins across epithelial cells to their main site of action. The polyimmunogobulin receptor pIgR is an Fc-receptor that facilitates the secretion of soluble polymeric isoforms of IgA and IgM, and is involved in the transcytosis and secretion of antibodies at mucosal surfaces [92]. The neonatal Fc–receptor (FcnR), facilitates transfer of maternal IgG to the fetus across the placenta [93,94], but in adult life it occurs in lungs, kidneys and other organs [95]. Fc-mediated transport into cells with various receptors can occur, and this could provide a means of autoantibodies accessing intracellular antigens (see Section 5).

3.3. Immune Complex Mediated Effects

In the examples above, in considering Fc-mediated secondary effects, the modes of action have assumed specific antibodies defined by their reactivity with particular autoantigens. However, tissue-damaging

effects can also be associated with Fc-mediated effects involving immune complexes that may not be as readily defined as antigen-specific. Soluble immune complexes associated with a variety of antigens have been particularly associated with the multi-system autoimmune diseases such as SLE, Sjögren's syndrome and rheumatoid arthritis, particularly those associated with various non-tissue-specific autoantibodies, and in these cases damage has been attributed to such immune complex-mediated effects. The secondary processes described above, associated with specific autoantigens are also relevant in these cases. Complement binding occurs as part of the normal physiological process, with sustained production of activated complement, and recruitment of phagocytes with cell-mediated tissue damage involving activated basophils and neutrophils.

Although each antibody involved in such complexes would be binding to a specific antigen, no single antigen has been associated with immune complex formation in any of these diseases. Immune complexes may be detectable in various tissues, and multiple autoantigens could contribute. For example, autoantibodies of multiple different specificities have been eluted from the immune deposits in glomeruli of patients with SLE and lupus nephritis, including autoantibodies to dsDNA, histones, C1q, Sm, SSA (Ro) and SSB (La) that are not specific to glomeruli, and are also present in the serum [96–98].

3.4. Therapies that Modify Fc-Mediated Secondary Effects of Antibodies

Although discussion of therapies for B-cell mediated pathogenic effects are often confined to those that directly impact on autoantibody levels, such as anti-CD20 (Rituximab), or plasmapheresis, it should be noted that immunotherapies that are directed at inflammation also directly impact on the Fc-mediated effects of autoantibodies. A variety of anti-inflammatory agents are routinely used. These include aspirin and other nonsteroidal anti-inflammatories (NSAIDs) that are inhibitors of cyclooxygenase (COX) enzymes COX-1 and COX-2, the enzymes that synthesize prostaglandins, reducing pain associated with inflammation, and glucocorticoids used for reducing inflammation. Also, new "biologics" such as anticytokine therapies reduce the activity of specific cytokines or their receptors, limiting the activation of monocytes, macrophages, and other effector cells [99]. An example is the success of drugs targeting the production of TNF by inflammatory cells in rheumatoid arthritis: these include Infliximab, Etanercept, Adalimumab, as first line drugs for treatment of patients with refractory rheumatoid arthritis [100].

4. Pathogenic Effects of Autoantibodies

As stated earlier, clear evidence for pathogenic effects of autoantibodies has been confined almost exclusively to those antibodies reactive with receptors on cell surfaces, adhesion molecules and ion channels or secreted products of cells. This section selects examples of such pathogenic autoantibodies and describes their injurious effects in the setting of disease.

4.1. Autoantibodies to Desmosomes and Hemidesmosomes

Autoantibodies to these cell-attachment proteins have been implicated in the development of bullous skin diseases characterized by epithelial separation (acantholysis) of mucous membranes, or mucous membranes and skin. These are represented by two major groups of autoimmune diseases, the pemphigus and pemphigoid syndromes (Table 1).

4.1.1. The Pemphigus Syndromes

The most direct evidence for pathogenic effects of Fab binding has come from studies of pemphigus vulgaris (PV), including paraneoplastic pemphigus associated with underlying neoplasms [101], and pemphigus foliaceus (PF), characterized by intra-epidermal blistering of skin and mucosa, with epidermal cell-to-cell detachment, and associated autoantibodies to desmogleins, the cadherin-type cell-cell adhesion molecules in desmosomes. In these diseases, there is clear evidence that the damage is Fab-mediated, that only some autoantibodies are pathogenic, and damage can be correlated with epitopes recognised. In PV, the acantholysis is mainly in the superbasilar region of the epithelial layer, and is strongly associated with autoantibodies to Dsg-3, when restricted to the mucous membranes. However, when the disease also affects skin, there are antibodies to Dsg-3 and Dsg-1. By contrast, autoantibodies in PF tend to be subcorneal, limited to the skin, and associated with autoantibodies to Dsg-1.

Each of these diseases can be reproduced by antibody transfer. In mothers with pemphigus there is placental transfer of autoantibody to the fetus, leading to transient PV in the neonate [9–11]. Both PV and PF can also be transferred to neonatal mice using IgG from patients [14,15,31,69]. In these studies, pathogenicity has been assessed by the development of skin blisters, but the effects can also be demonstrated *in vitro* using cultured human keratinocytes [18]. The effect was Fab-mediated, with no requirement for Fc interactions, as passive transfer to mice occurred using $F(ab)_2$, Fab or scFv [31,69–71]. Ultrastructural studies in mice showed that antibody penetrated desmosomal plaques, blocking cell-cell interactions within the desmosomes and resulting in basal cell keratinocyte separation and development of a "tombstone-like" appearance, with split desmosomes with anti-Dsg3 IgG bound extracellularly [102]. The process occurred rapidly, within hours, without inflammation and appeared to be a direct result of antibody binding [103]. These results are consistent with the observation that in human PV there is rarely any inflammation in the lesions, no requirement for complement, and autoantibodies in the human diseases are predominantly IgG4.

Epitope analysis performed using polyclonal antibodies [104,105], human mAb [106,107] and panels of human anti-Dsg scFv isolated by phage display from a patient with active acute mucocutaneous PV [70] have shown that antibodies bind to all regions of the extracellular domains of Dsg1 and Dsg3. Both pathogenic and non-pathogenic mAb and Scv have been isolated, and there is an immunodominant epitope region at the N-terminus that correlated with pathogenicity. The epitope(s) recognised in polyclonal sera are predominantly conformational and stabilised by calcium [108], but although most Scv isolated reacted similarly, there was variability, both in capacity to react by immunoblotting, and to show cross-reactivity between Dsg1 and Dsg3. Genetic analysis of these Scvs showed restricted patterns of heavy and light chain gene usage that differed according to the Dsg recognised.

4.1.2. The Pemphigoid Syndromes

These are blistering skin diseases in which the site of the lesion is at the dermal-epidermal junction, and include bullous pemphigoid (BP), pemphigoid gestationis (PG) and epidermolysis bullosa acquisita (EBA). BP is characterized by skin lesions appearing as areas of urticaria that develop into large bulla, with separation of the epidermis from the dermis, with an intense infiltration of inflammatory cells in the underlying dermis, and destruction of components of the hemidesmosome and extracellular

matrix [26]. PG also known as herpes gestationis, is a non-viral variant of BP that occurs in early pregnancy or post-partum and can result in transient neonatal PG [21,22]. In BP and PG, autoantibodies are directed to hemidesmosomes that are cellular structures involved in anchoring keratinocytes to the basement membrane, and the major autoantigen is BP180, a transmembrane protein belonging to the collagen family, also known as collagen XVII. The extracellular domain of BP180 contains 15 collagen domains, interrupted by 16 non-collagen (NC) sequences, and the membrane-proximal NC domain NC16A contains multiple epitopes recognised by sera from patients with BP [109–112].

Epidermolysis bullosa acquisita (EBA) is another subepithelial blistering disease in which blisters are formed as a result of detachment of the epidermis from the sub-basal lamina densa, often in areas of trauma. It is associated with autoantibodies to the N-terminal non-collagenous domain (NC1) of type VII collagen that is the major component of anchoring fibrils in the basement membrane. It has similarities to dystrophic epidermolysis bullosa in which there is a hereditary defect in the gene that encodes type VII collagen, and to BP, although the degree of associated inflammation is more variable, and may be less than that seen in BP [113].

As the human autoantibodies to both BP180 (collagen XVII) and collagen VII react poorly with the animal antigens due to sequence differences, there is limited evidence for direct passive transfer of either BP or EBA to animals by human IgG [23,31,114,115]. However, in each case, passive transfer has been achieved using antibodies specific to the pathogenic epitope region of the recipient animal. Thus, although autoantibodies to human NC16A do not react with NC16A from other species, mouse models of BP have been derived in which passive transfer of antibodies from rabbits or sheep immunized with recombinant mouse NC16A induce blistering skin lesions in neonatal or adult mice [24,27]. Similarly, an animal model of EBA was developed using passive transfer of rabbit antibodies to the mouse NC1 domain [31].

The mechanisms of damage in BP or in EBA are more complex than those in PV or PF, requiring interactions through the Fc region of the molecule to trigger interactions with the innate immune system, involving complement and inflammatory cells. *In vitro* in both diseases, human autoantibodies induced epidermal-dermal separation in cryosections of human skin that did not take place with $F(ab)_2$, and the process required the addition of neutrophils [31,32]. Experiments *in vivo* have confirmed these results, and have demonstrated the role of neutrophil elastase and of mast cells in these processes [25,26].

Notably, in both the pemphigus and pemphigoid syndromes, the epitopes recognised and IgG subclasses of autoantibodies are important for pathogenesis. Both pathogenic and non-pathogenic autoantibodies can occur, and pathogenic autoantibodies can react with various subregions within an epitope region, giving an additive effect when several different autoantibodies are combined [16,17,116]. Also, the IgG subclass is important. None of the autoantibody responses are restricted to a single subclass, and the complexity of the antibody responses has been extensively reviewed [117]. However, IgG4 which has limited capacity to activate Fc receptors, and does not activate complement is particularly associated with PV [118,119]. By contrast, both IgG4 and IgG1 are associated with BP, and IgG1 has been associated with the acute phase, whereas IgG4 was more associated with remission [120].

4.2. Autoantibodies to H^+/K^+ATPase and Gastric Intrinsic Factor

Autoimmune gastritis is a chronic inflammatory condition of the parietal cell-rich area of the stomach that may, over a period of 20–30 years, lead to gastric atrophy associated with malabsorption of cobalamin (vitamin B12), and development of pernicious anemia. The two autoantibodies implicated in the autoimmune process react with gastric intrinsic factor that is synthesized by the parietal cell, and with H^+/K^+ ATPase that is also synthesized by the parietal cell and is responsible for the acidification of the gastric juice [121,122].

Gastric intrinsic factor (IF) is a glycoprotein that is required for absorption of cobalamin in the small intestine, and two autoantibodies of different specificities are reactive with it. One is a blocking antibody reactive with the receptor site for the binding of cobalamin to intrinsic factor [123], and the second is reactive with a site remote from the cobalamin binding site, and does not interfere with the formation of the intrinsic factor-cobalamin complex, but blocks the complex from binding to the cubam receptor in the ileum [124,125]. Antibodies have been demonstrated in gastric juice in complex with intrinsic factor [125,126], and their presence could explain the malabsorption of cobalamin and development of pernicious anemia. Normally a large excess of intrinsic factor is secreted, and is available for binding with cobalamin. Any antibody present would readily dissociate in the acidic milieu. However, in the achlorhydric gastric juice of autoimmune gastritis binding is increased (100% at pH 7.5 *vs.* 30% at pH 3.2 at 90 s) [126], thus reducing the availability of cobalamin for absorption in the ileum where the second antibody inhibits binding to the cubam receptor.

The other major parietal cell autoantigen has been identified as H^+/K^+ ATPase, an integral membrane proton pump. Parietal cell antibodies have been shown to deplete H^+/K^+ ATPase activity from parietal cell membranes [39], and their presence is associated with raised levels of gastrin and hypochlorhydria [127] favouring the intrinsic factor antibody-cobalamin reaction. In rats, repeated infusions of IgG from sera of subjects with pernicious anemia with gastric atrophy and parietal cell antibodies resulted in thinning of the gastric mucosa, reduction in parietal cell mass and hypochlorhydria [38]. Furthermore, in women with antibodies to H^+/K^+ ATPase, IgG was demonstrated on the surface and within parietal cells that showed no sign of atrophy of the cell or the oxyntic gland containing them suggesting that antibodies have access to H^+/K^+ ATPase [127] and may be involved pathogenetically in the failure of the proton pump.

Although these autoantibodies are associated with the development of autoimmune gastritis, and are predictive of the cobalamin malabsorption that leads to the development of pernicious anemia [128], it remains generally accepted that experimental autoimmune gastritis is primarily a T-cell mediated disease [129,130]. Nonetheless, taken together, these two autoantibody populations could explain the symptoms and progression of disease in autoimmune gastritis. Atrophy of parietal cells, and loss of function associated with parietal cell antibodies would establish conditions for depletion of intrinsic factor, both directly by lack of secretion, and also by creating conditions within the stomach that are favourable to antibody binding.

4.3. Autoantibodies to Collagen

Rheumatoid arthritis illustrates the complexity of pathogenic antibody responses in a single disease, in which the secondary pathogenic effects associated with Fc binding play a major role, but may be

modified by Fab epitope reactivity. It is generally considered to be immune complex-mediated. The primary target of the disease is the articular joint, with cartilage destruction, bone erosion, and an inflammatory infiltration within the synovium. It is characterized by three populations of autoantibodies: antibodies to the Fc-region of IgG (rheumatoid factor, RF), antibodies to citrullinated proteins (ACPA), and antibodies to type II collagen (CII). Of these, the presence of RF and ACPA predict more severe erosive disease [131], but CII is the only joint specific antigen, and autoantibodies to CII are frequent early in the disease [132–135].

A similar arthritis, collagen antibody induced arthritis (CAIA) can be transferred passively to naïve mice using CII-reactive mouse serum [136], human serum [56], or mouse mAb to CII [57,67]. As CAIA is not MHC-restricted, and can be induced in mice lacking an adaptive immune system, it is an excellent model to examine the effector role of autoantibodies in the induction of arthritis [57]. The arthritogenic mAbs to CII are reactive with conformational epitopes on the CII triple helix, several of which are shared with human antibodies (Table 3).

The pathogenic mAbs used to induce CAIA bind to the surface of the cartilage and C3 is deposited at the same site [137,138]. Histologically, there is inflammation associated with heavy infiltrations of neutrophils along with bone and cartilage erosion, pannus formation, and fibrin deposition [57]. The inflammation that results can be considered as a secondary effect of the development of immune complexes within the cartilage, in a process that requires proinflammatory cytokines, particularly TNF and IL1β, Fcγ receptors, and complement activation and C5a [57,138–142]. Neutrophils are essential not only for arthritis development, but also for its maintenance [140]. C5a is a potent neutrophil chemoattractant, and mice that lack C5a receptor do not develop CAIA [143]. These processes are similar to those seen in other animal models of immune complex mediated arthritis, and are consistent with changes seen in a subset of patients with human RA who have high levels of antibodies to CII [144–146]. It is likely that immune complexes containing RF or ACPA exert pathogenic effects within the joint by similar mechanisms.

Table 3. Mouse mAbs to CII used for mapping and defining epitopes in collagen antibody induced arthritis (CAIA) [1].

MAb	CIIC1	UL1	M2139	CIIF4
IgG subclass	IgG2a	IgG2b	IgG2b	IgG2a
Epitope location on CII (amino acids) [147]	356–369	494–504	551–564	926–936
Sequence [147,148]	ARGLT	LVGPRGERGFP	MPGERRGAAGIAGPK	HRGFT
Possible binding site on collagen	Chondroadherin	Integrin	Collagen IX/integrin	Stromelysin
Arthritogenic *in vivo* (mouse)	Yes	Yes	Yes	No
Antibodies in human arthritis	Yes, RA > OA	Yes, severe RA	Yes in RA (less frequent)	Yes, OA > RA
Effects on fibrillogenesis *in vitro* [149]	Inhibition	Not tested	Inhibition	No effect
Effects in chondrocyte cultures [58,59,62]				
Chondrocytes	Normal	Vacuolated	Pleiomorphic	Normal
Collagen fibrils	Thin	Normal	Thick, aggregated	Normal
Matrix synthesis	Increased	Normal	Normal	Normal
Effects in cartilage cultures [60,62]				
Proteoglycan loss	Yes	Yes	Yes	No
Collagen denaturation	Yes	Yes	Yes	No
Collagen loss	Yes	Yes	Yes	No

[1] modified from [150].

In addition to these changes, autoantibodies to CII have effects that differ according to the epitope specificity of the mAb, and although various mAbs can induce arthritis when injected at low doses [137], enhancement by a large dose of a single mAb does not induce the severe arthritis that can be induced by combinations of mAb with different epitope specificities [151,152]. There are striking differences in the induction of CAIA by mAb recognising different epitopes that cannot be related to the affinity or the subclass of the antibody [151,152] (Table 3). Antibodies reactive with the epitope recognised by mAb UL1 are associated with chronic, relapsing arthritis in mice, rats and humans [62,153], whereas another mAb CIIF4, reactive with the F4 epitope on CII, is non-arthritogenic in mice, and inhibits CAIA development when given with known arthritogenic mAb [154]. Antibodies reactive with the F4 epitope have been associated with osteoarthritis in humans [154].

Several mAb have been shown to modify cartilage synthesis or breakdown *in vitro*, in the absence of immune cells or complement. Two arthritogenic mAb, CIIC1 and M2139 inhibited fibrillogenesis of CII *in vitro*, whereas the non-arthritogenic mAb CIIF4 did not [149]. The arthritogenic mAb, CIIC1, M2139 and UL1 also impaired cartilage formation by cultured chondrocytes, causing morphological changes to the cells, or disorganization of the cartilage fibrils that differed according to the mAb [58,59,62], as well as proteoglycan loss and cartilage damage in cartilage explant cultures [60,62]. In these cultures, $F(ab)_2$ was as potent as the mAb itself [60], and the location of the epitopes for these arthritogenic mAb suggested that the degradative effects observed were related to interference with interactions between CII and other essential matrix components. Viable chondrocytes appeared to play a protective role, as the matrix damage was substantially increased when chondrocytes were pre-killed by freeze-thawing the cartilage before culture [61]. Interestingly, the non-arthritogenic mAb CIIF4 had no apparent effects when tested in the cultures alone, but the addition of CIIF4 to cartilage explants cultured with arthritogenic mAb had a clear and unexplained protective effect on the cartilage matrix [61].

Notably, this discussion has considered only one potential autoantibody, although it is the one with joint specificity. ACPA are reactive with neo-epitopes found on proteins in which citrulline has been derived by deimination of arginine by peptidyl-arginine-deiminases (PADI), an enzyme found in the inflamed synovium [155,156]. There is an HLA association of RA with HLA-DRB1 genes, particularly HLA-DR4 and DR1 alleles containing a "shared epitope" [157]. One scenario could be that the primary autoantigen that provides the joint specificity is CII, leading to immune complex deposition and low-grade inflammation. Within that milieu, joint antigens are citrullinated by PADI in macrophages in the initial CII-immune complexes. Complement activation in immune complexes has been shown to enhance phagocytosis, antigen presentation and B cell activation [158] and this could lead to increased ACPA production and enhanced immune complex formation. RF may be a secondary effect of enhanced antigenic stimulation, as it can occur in subjects with chronic infections like parasitic infections or in healthy subjects after multiple vaccinations, and may potentiate the inflammation [159]. Consistent with this, is our observation that in early RA, ACPA or RF were rare in the absence of anti-CII, and that the presence of antibodies to CII in the absence of RF or ACPA was associated with a more favourable outcome.

4.4. Autoantibodies to Muscarinic Receptors

Sjögren's syndrome is a chronic inflammatory disease, characterized by lymphocytic infiltration of the salivary and lacrimal glands, leading to the characteristic symptoms of a dry mouth and dry eyes and

it may also affect other exocrine glands [160]. It may occur alone (primary Sjögren's Syndrome, SS), or with other autoimmune diseases (secondary SS). Primary SS is characterized by hypergammaglobulinemia, rheumatoid factor, and autoantibodies to two ribonucleoprotein antigens, SS-A (Ro) and SS-B (La). Of these autoantibodies, anti-La is the most abundant, occurring at high frequency, and constituting up to 10% of serum IgG [161]. Secondary SS although sharing the same sicca manifestations of primary SS, does not have the same clinical and genetic features and, serologically, the antibody profile takes on that of the associated disease be it rheumatoid arthritis, SLE or primary biliary cirrhosis for example. The contribution of these antibodies to the development of Sjögren's syndrome is unknown. However, recently there has been increasing evidence that the secretory deficit is related to the presence of a population of autoantibodies that target the M3 muscarinic acetylcholine receptor (M3R) in the salivary glands. M3R mediates cholinergic neurotransmission in tissues innervated by the autonomic nervous system such as salivary and lacrimal glands, blood vessels, the bladder and the gastrointestinal tract, and plays crucial roles in exocrine secretions and in smooth muscle contraction, including gastrointestinal motility.

The first evidence for such autoantibodies came from the observation that IgG from patients with primary SS caused loss of secretory function of exocrine tissues in the NOD mouse [40], and activated muscarinic acetylcholine receptors of rat parotid gland independent of anti-Ro or anti-La [162]. In a further study using a functional smooth muscle bioassay to demonstrate antibodies to M3R, 5 of 9 patients with primary SS and 6 of 6 patients with SS and rheumatoid arthritis were positive. By contrast, sera from 15 of 16 controls, including healthy individuals, patients with rheumatoid arthritis without sicca, and patients with systemic lupus erythematosus, were negative [163]. More recently, the presence of autoantibodies has been confirmed in both primary and secondary SS using a variety of functional assays based on measurement of contractions in smooth muscle [41,42,164], or of Ca^{2+} influx in salivary cells [165] and also ELISAs based on peptide antigens [166–168]. The effect is Fab-mediated, and is induced by monovalent Fab, $F(ab)_2$ or IgG [42]. Various epitopes have been mapped on the extracellular loops of the receptor, and antibodies binding to these epitopes may have different effects according to the location of binding [166].

M3R antibodies have also been associated with altered gastrointestinal mobility in Sjogren's syndrome [169]. Functional antibodies that affect parasympathetic neurotransmission could not only lead to altered secretion by exocrine organs such as salivary and lacrimal glands, but also mediate the bladder and gastrointestinal dysfunction in SS by altering the effects of endogenously released acetylcholine on smooth muscle and explain the link between the two clinical entities.

5. Pathogenic Effects of Autoantibodies to Intracellular Antigens

The pathogenic autoantibodies that we have considered so far (Table 1) have been reactive with autoantigens that are exposed to the blood and body fluids and are freely available to antibody in the contact medium. By contrast, the intracellular autoantigens may be separated from the extracellular milieu by several membranes. Many are shared by all nucleated cells, and are widely distributed within cells, being located in ribosomes, mitochondria, centromeres, nucleoli, and nuclei, and even on cellular structures present only transiently during cell division. Others may be quite tissue or organ specific such as the autoantibodies associated with paraneoplastic neurological syndromes that target nuclear or

cytoplasmic intracellular proteins in the brain [170], or the autoantibodies to the enzyme glutamic acid decarboxylase (GAD) that occurs in β-islet cells in the pancreas and GABA-producing cells in the brain. If the antigen has been identified, functional impairment may have been demonstrated *in vitro* by autoantibody so the potential pathogenicity of these autoantibodies *in vivo* cannot be dismissed [171–175].

How autoantibody gains access to intracellular antigens is still unclear. However penetration of cells by autoantibodies has been described for various intracellular antigens, including nuclear antigens RNP [176], Ro and La [177,178] and double-stranded DNA (dsDNA) [179–183], as well as ribosomal P proteins [184], the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2) located in mitochondria [185], and neuronal and retinal proteins [186,187]. Such penetration has been associated with functional changes that may differ according to the phase of the cell cycle or state of activation of the particular cell [177,181,188]. Also antibody has been shown to lead to apoptosis of the transfected cells [181,186,187,189].

The methods of penetration differ for different antibodies. Internalization mediated by binding to Fc-receptors has been described for autoantibodies to nuclear antigens, such as anti-RNP, anti-Ro and anti-La that bind to Fcγ receptors [177–179], or the IgA antibodies to PDC-E2 in primary biliary cirrhosis that bind to the polyimmunoglobulin receptor pIgR for penetration of biliary epithelial cells [185]. Fc-mediated internalization of autoantibodies may be of particular significance in the brain, where Fc receptors are widely distributed in neural tissue, increase during aging, and may be involved in neurological disorders [91,190]. Fc-dependent uptake of IgG has been shown by neurons, particularly Purkinje cells, with accumulation in the cytoplasm and microtubular transport within the cell [90,191–195].

Not all mechanisms for cell penetration depend on binding to Fc-receptors. One method that has been extensively studied for autoantibodies to dsDNA depends on involvement of arginine-rich peptide sequences. Panels of mAbs that react strongly with anti-dsDNA have been derived from the spleens of (NZBxNZW)F1 mice [196]. Although mAbs alone have been shown to enter and accumulate within the nucleus in a few hours, as well as mAb, or $F(ab)_2$ or F(ab) coupled to biotin, fluorescein or peroxidase, not all anti-dsDNA showed equal cell penetration and translocation. Penetration and translocation were linked to increased lysines and arginines in the complementarity determining regions (CDR) of the antibodies, and peptides corresponding to CDR2 linked to CDR3 of several penetrating mAb were designed that penetrated cells, and could be used as a vector to transport macromolecules[180,196]. Further studies showed that this was associated with electrostatic interactions of arginine residues in the CDR interacting with negatively charged sulphated polysaccharides on the cell surface [183], and arginine-rich cell penetrating peptides are now being investigated as promising tools for precise intracellular drug delivery [197,198].

These arginine-rich cell-penetrating sequences may also contribute to the pathogenicity of anti-dsDNA in the development of lupus nephritis. Anti-dsDNA occur in 50%–70% of patients with SLE, and are strongly associated with lupus nephritis and a rise in titre of anti-dsDNA can be indicative of a flare of disease activity. However, not all anti-dsDNA are pathogenic and not all patients with high levels of anti-dsDNA have lupus nephritis. Statistical analysis of sequences of pathogenic human IgG mAbs to ds-DNA from patients with SLE showed multiple somatic mutations in the variable regions of the antigen, particularly an accumulation of arginines in the CDR that were predicted to interact and enhance binding with dsDNA. This was confirmed by cloning and expressing variant IgG molecules to localise particular arginine residues that were critically sensitive for the ability to bind DNA [199].

The apoptosis that may occur following cell penetration by anti-dsDNA and other autoantibodies may also play a role in the pathogenicity of autoantibodies to intracellular antigens. Cellular apoptosis may not only provide a source of autoantigens that could drive the expansion of the immune repertoire, but may also cause tissue injury or may modulate the immune response [200,201]. On the right genetic background, an autoantibody to a widely dispersed autoantigen, such as the nuclear antigens, that has the capacity to cross cell membranes and induce apoptosis could be the trigger for a greatly enhanced immune response to neoepitopes generated within the dying cells, but also release of the same cellular components to form immune complexes that could contribute to immune-mediated damage.

6. Evidence for Pathogenicity of Autoantibodies to Intracellular Antigens

In contrast to the previous examples of pathogenic autoantibodies to cell surface and extracellular antigens, there has been very little direct evidence that autoantibodies to intracellular antigens have any direct pathogenic effect. It has been assumed that pathogenesis is primarily T-cell mediated in the autoimmune diseases in which they occur, and the autoantibodies are merely markers of an autoimmune response. In this context, a recent study of uptake of autoantibodies to the cytoplasmic autoantigen Yo in Purkinje cells is of particular interest [193]. Anti-Yo is associated with paraneoplastic cerebellar degeneration associated with gynaecological and breast malignancies and is representative of a larger group of autoantibodies in paraneoplastic neurological syndromes targeting intracellular proteins [170]. The role of these autoantibodies has been unclear, as autoantibody levels do not correlate with disease severity, and prognosis is poor despite immunotherapies [202]. However, anti-Yo was shown to penetrate Purkinje cells, accumulate intracellularly, bind to the Yo antigen, and cause cell death, providing direct evidence that the autoantibodies may be directly pathogenic [193].

6.1. Autoantibodies to Glutamic Acid Decarboxylase

These autoantibodies represent an interesting example where two different populations of autoantibodies to the same intracellular antigen are associated with different diseases affecting different tissues. Glutamic acid decarboxylase is a pyridoxal-5'-phosphate (PLP) dependent enzyme that catalyses the production of the inhibitory neurotransmitter γ-amino butyric acid (GABA) from L-glutamate in the brain and in pancreatic islet β-cells where GABA has a paracrine function. It occurs as two isoforms, GAD65 and GAD67, GAD65 being most frequently autoantigenic. Autoantibodies to GAD65 occur in the serum of 70%–80% of subjects with type 1 diabetes [203,204], and in certain neurological diseases, including Stiff Person Syndrome (SPS) characterized by progressive disabling muscle rigidity and spasms [205,206], but also in cerebellar ataxia, epilepsy, and various other disorders that may be related to altered GABA production in the central nervous system [207,208]. About 30%–50% of patients with GAD-related neurological diseases have diabetes, but SPS is rare in type 1 diabetes. Table 4 summarises the different autoantibody responses in type 1 diabetes and GAD-related neurological diseases that may be related to the structure and function of the two isoforms.

Table 4. Comparison of autoantibodies to glutamic acid decarboxylase (GAD) in type 1 diabetes and SPS.

	Type 1 Diabetes	**SPS**
Autoantibody levels: serum	Low to moderate [209–211]	moderate to very high [209,210,212,213]
CSF	Not detected	oligoclonal [209,212,213]
Frequency: anti-GAD65	70%–80% [214,215]	70%–80% [217,218]
Anti-GAD67	12% cross-reactive [216]	50%–60% [217,218]
Immunofluorescence [1]		
Pancreas	GAD65 [219]	GAD65 only [219]
Brain	Minimal [219]	GAD65, 67 [219]
Enzyme inhibition	2% [217]	60% [210,217]
Western blotting:	<10% [219–221]	GAD65 only [222]
B cell epitopes:		
GAD65 conformational	Catalytic region [223]	Catalytic region [222]
GAD65 N-terminal linear	None ascertained [210,224]	Amino acids 4–22 [210,224,225]
GAD65 C-terminal linear	Rare	Amino acids 475–585 [222]
GAD67 conformational	GAD67 or GAD65 [216]	GAD67 specific [210,224]
Cross-inhibition (see text)	b96.11 > b78 [226]	b78 > b96.11 [210]
Anti-GAD65 transfer to animals (see text)	Diabetes: no Neurological: yes [227–231]	Not recorded
Other autoimmune diseases	Thyrogastric cluster [232]	Diabetes 30%–60% Thyrogastric cluster [207,208]
IvIg	……..	Effective [234,235]
Plasmapheresis	No effect [233]	……..
Rituximab	……	Varied [236–240]

[1] Defined by inhibition with recombinant GAD65 and/or GAD67.

The two isoforms of GAD are structurally very similar consisting of a highly conserved catalytic domain, with an N-terminal regulatory sequence of about 100 amino acids that differs substantially in sequence and represents the membrane-binding domain that co-localises GAD65 with the GABA-transporter on the membranes of synaptic vesicles [241,242]. There is no structural information available on this, but crystal structures have been derived for the N-terminally-truncated GAD65 and GAD67 catalytic domains, and these domains retain full enzymatic activity [243]. The two have different functions within the cell. GAD67 is continuously active, continuously produces GABA at a low level, and is predominantly cytoplasmic, whereas GAD65 occurs in the cell primarily as apoenzyme and provides an increased level of holo-enzyme in circumstances that demand a rapid surge of GABA synthesis and release, a process that involves translocation of GAD65 to the membrane of synaptic vesicles [242–244].

In type 1 diabetes, antibodies to GAD are almost entirely anti-GAD65: antibodies to GAD67 occur in only 10%–12% of patients, and represent a cross-reactive sub-population of anti-GAD65 [216]. The autoantibodies recognise highly conformational epitopes on GAD65 within the conserved catalytic domain, but not in the N-terminal membrane binding domain [223]. Despite reactivity with conformational epitopes in the catalytic domain, diabetes-associated autoantibodies to GAD are rarely enzyme inhibitory [217].

By contrast, in SPS and other anti-GAD-positive neurological diseases, very high levels of autoantibodies to both GAD65 and GAD67 occur, the autoantibodies can be detected by immunoblotting [245] and most sera are strongly enzyme inhibitory. Although sera from patients with SPS or other anti-GAD-positive neurological diseases, and sera from type 1 diabetics recognise epitopes across the catalytic domain of GAD65, an additional linear epitope in the N-terminal membrane-binding domain is present in SPS but not in type I diabetes [210,224,246].

In anti-GAD-positive neurological diseases, both anti-GAD65 and anti-GAD67 occur in the cerebrospinal fluid (CSF). Although the levels of antibodies are much lower than in serum they represent a proportionally greater fraction of the total IgG, are often oligoclonal or monoclonal IgG antibodies, and may be produced intrathecally [209,211]. A comparison of anti-GAD65 and anti-GAD67 in paired samples of serum and CFS from 106 patients with anti-GAD-positive neurological conditions showed different responses in the two locations. Anti-GAD65 was present in all sera and anti-GAD67 was present in all CSF. However, there were 9 paired samples in which the CSF was positive for anti-GAD67 but the serum was negative [245].

Further evidence for differences in reactivity between type 1 diabetes and anti-GAD positive neurological diseases has come from epitope-mapping studies using recombinant Fab (rFab). Various human GAD-65-specific mAbs have been derived from patients with newly diagnosed type 1 diabetes or autoimmune polyendocrine syndrome 2 [247–250], and rFab has been prepared from several of them [226]. Although all of the mAb reacted with GAD65 only, and recognised multiple conformational epitopes in the catalytic domain, cross-inhibition studies with these rFab have revealed differences in the patterns of epitopes recognised in type 1 diabetes and SPS. In particular, antibodies reactive with an epitope defined by mAb b96.11 occurred in most patients with type 1 diabetes [226], whereas antibodies reactive with an epitope defined by mAb b78 were more closely associated with SPS, and mAb b78 inhibits the enzyme [210]. The b96.11 and b78 epitopes are predicted to be on opposing faces of the crystal structure [251]. The b78 epitope is in the C-terminal region that moves during catalysis, and co-locates with the N-terminal residues (from amino acid 84) in the crystal structure, close to the membrane binding domain. Moreover, in support of this, mAb b78, but not mAb b96.11 has been shown to disrupt the association of GAD with GABA-containing synaptic vesicles from rat brain *in vitro* [230]. Also, the human mAb b78 and to a lesser extent b96.11, have been shown to cause neurological changes when infused into the brains of animals [230]. Thus, there may be a major epitope region specifically associated with anti-GAD positive neurological diseases where autoantibodies could interfere with the GABAergic system in the central nervous system not only by direct inhibition of GABA synthesis, but also by interfering with membrane binding of GAD65 with the GABA transporter.

The question remains, whether these autoantibodies to intracellular antigens are pathogenic. Type I diabetes is generally considered to be primarily T-cell mediated. However, anti-GAD65 may be present in serum many years before the development of disease, and may predict progression to diabetes [252]. Although there is little evidence that autoantibodies to this important intracellular autoantigen are pathogenic *per se*, their role in the disease is yet to be elucidated.

In the case of SPS and other neurological diseases, there is more evidence that symptoms are antibody-mediated. *In vitro*, IgG from serum containing high levels of antibodies to GAD from patients with cerebellar ataxia have been shown to suppress GABA release in cerebellar slice preparations [253–257]. *In vivo*, infusions of IgG containing anti-GAD65 from patients with various neurological diseases have

been shown by electrophysiology and neurochemistry to alter cerebellar activity, impair learning, and affect spinal cord activity in rats [231]. SPS has been successfully treated with intravenous Ig, and is the treatment of choice for intractable SPS [234,235], suggesting that antibodies may be important in pathogenesis. Although both GAD65 and GAD67 are intracellular antigens, the mAbs b78 and b96.11 have been shown to penetrate the AF5 rat CNS cell line [228], possibly being internalised by Fc receptors that are widely distributed in neural tissue including Purkinje cells [192,193]. As Purkinje cells are the major source of GABA in the brain, uptake of anti-GAD could disrupt function in these cells.

6.2. Autoantibodies to the E2 Subunit of the Pyruvate Dehydrogenase Complex (PDC-E2)

Primary biliary cirrhosis (PBC) is a rare chronic liver disease characterized by progressive obliterative cholangitis, with liver destruction and cirrhosis in which there is a strong diagnostic association with anti-mitochondrial autoantibodies to intracellular enzymes, the 2-oxoacid dehydrogenase complexes (OADC) that are essential enzymes in energy metabolism. The most frequent reactant is the E2 subunit of the pyruvate dehydrogenase complex, dihydrolipoamide acetyltransferase that has lipoic acid covalently attached as co-factor. Autoantibodies to E2 subunits of the related enzymes, 2-oxo-glutarate dehydrogenase complex (OGDC-E2) and the branched chain 2-oxo-acid dehydrogenase complex (BCOADC-E2) that share similar immunodominant epitope regions in the inner lipoyl domains of the three enzymes occur less frequently [258].

Anti-mitochondrial antibodies occur in sera many years before the development of symptoms, and are highly predictive of progression to primary biliary cirrhosis [259–262]. However, these enzymes are found throughout the body, yet the disease is limited to the biliary epithelial cells. An immunodominant epitope region has been identified in the inner lipoyl domain of PDC-E2, and initial tolerance breakdown has been attributed to xenobiotic modification of the lipoic acid cofactor causing cross-stimulation with a neo-epitope and epitope spreading to self-antigen in genetically susceptible individuals [263], but the autoantibodies associated with disease development are reactive with human PDC-E2, and not a neo-epitope [264]. Sera from patients with primary biliary cirrhosis contain very high titers of these autoantibodies, particularly anti-PDC-E2, and the autoantibodies are strongly enzyme inhibitory *in vitro* [171,173,265,266]. However, although primary biliary cirrhosis may recur after liver transplant, and all patients continue to produce high levels of autoantibodies, levels of serum autoantibodies have not been linked to recurrence of disease, and there is no direct evidence that the antibodies are pathogenic *in vivo*.

A possible clue to pathogenesis is the observation that PDC-E2 may be detected on the apical surface of biliary epithelium in patients with primary biliary cirrhosis, including some with donor livers [267,268]. Biliary epithelial cells have the capacity to export dimeric IgA to the bile by transcytosis, a process involving the polymeric immunoglobulin receptor (pIgR). Thus, the tissue specificity of the damage may be related to IgA anti-PDC-E2 transcytosed for secretion through the biliary epithelium that could carry PDC-E2 to the cell surface in the process. IgA antibodies have been described in in bile, saliva and urine in primary biliary cirrhosis [269–272] and the presence of IgA anti-PDC-E2 in serum and in saliva, has been linked to the histological progression of PBC [273].

Support for this hypothesis has come from studies of transcytosis of IgA into Madine-Darby canine kidney (MDCK) cells transfected with the human pIgR, in which IgA derived from patients with PBC

penetrated the cells, colocalised with PDC-E2 within the cell [185], and induced caspase activation indicative of apoptosis [274]. Enhanced caspase activation was limited to patients with anti-PDC-E2 IgA expression, and required pIgR expression. Moreover, there was a strong correlation between the level of anti-PDC-E2 IgA and the degree of caspase activation [274]. Patients with PBC may complain of dry eyes and dry mouth, a feature of Sjögren's syndrome that is known to coexist with PBC [275]. As the pIgR is also found in the lacrimal and salivary glands, transcytosis of PDC-E2 could occur there also as abnormal apical staining of PDC-E2 has also been shown in salivary glands in patients with coexistent Sjögren's syndrome and primary biliary cirrhosis [276].

The importance of transcytosis of IgA anti-PDC-E2 in the development of liver damage and the sicca syndrome remains to be determined. IgA anti-PDC-E2 could play a direct role in pathogenesis by binding and inhibition of enzymes during transcytosis in the cell [272] or by depleting energy levels by transport of newly synthesized PDC out of the cell [270], or by apoptosis [263,277,278]. Although apoptotic bodies have been linked to increased antigen presentation and tolerance breakdown leading to autoimmunity, apoptosis has also been described as an outcome of cell penetration by antibodies, and interference with intracellular functions [177,181]. If this is the case, cell transport by the pIg receptor could provide both the initial stimulus for apoptosis of the biliary epithelial cells and massive amplification and ongoing antigenic stimulation when T-cell mediated processes become involved.

7. Conclusions

Autoimmune diseases are polygenic diseases, with more than 200 loci linked to their development, many of which are associated with the major histocompatibility complex and with particular pathways of inflammation or acquired or innate immunity that culminate in disease [279,280]. By the time the disease presents the situation is already complex as a result of many of these pathways. Both CD4 and CD8 T cells are involved, as well as various cells and cytokines associated with inflammation. One or several autoantibodies of different specificities may be present in the one disease and T-cell mediated pathogenic effects may also be operative. In this review we have been selective in our choice of autoantibodies to illustrate the pathogenic role they may have in autoimmune disease. However, we acknowledge they are only part of an extensive and expanding repertoire of autoantibodies of novel specificities, such as those associated with severe debilitating diseases of the nervous system [281]. Moreover, various autoantibodies have prognostic significance, such as antiphospholipid antibodies in SLE that may be associated with a more severe clinical outcome including more frequent renal disease, CNS involvement, thrombocytopenia and clotting events, or the autoantibodies to neuronal antigens associated with paraneoplastic syndromes [281].

Studies of mAbs have shown that not all autoantibodies contributing to a polyclonal response are equally pathogenic, although pathogenicity may be enhanced by combinations of mAbs. Differences in pathogenicity may be related to Fab-mediated diversity in epitope specificity, or to Fc-mediated effects based on class or subclass of antibody that include activation of complement and recruitment of effector cells common to pathways of both acquired and innate immunity. Moreover, pathogenic autoantibodies require access to the target autoantigen to exert a damaging effect. This is not only true for autoimmune diseases of the central nervous system that is protected from serum autoantibodies by the blood brain barrier, but also for autoantibodies to intracellular antigens such as dsDNA that require poly arginine

peptide sequences in their antigen-binding domain to cross the cell membrane, and for antibodies to PDC-E2 that access biliary epithelial cells by the pIgR.

The initiation of the autoimmune response may occur long before symptoms appear as observed in population studies where autoantibodies have been detected in apparently healthy subjects many years before overt disease. Nonetheless, at this point a damaging autoantibody may be already exerting its pathogenic effect, and the outcome depends on a balance between damage and repair. This is exemplified by the damage to the extracellular matrix in primary cultures of bovine cartilage induced by mAb to CII where damage is much less in the presence of living chondrocytes responsible for its synthesis and repair than in their absence [61]. Over time, those damaging effects may be small but significant, given that there will be a balance between antibody-induced destruction and on-going cell-mediated repair, and this balance will depend on the level and pathogenicity of the antibody and the vitality of the cell.

It is plausible that many autoantibodies initiate the damage that leads to autoimmune diseases, and this damage is enhanced by the same T cell activation and inflammation that amplify normal responses to foreign antigens. In that case, the response amplified by antibodies, cytotoxic T cells and inflammation results in the elimination of the foreign antigen and the process subsides. In the case of an autoantigen, the antigen is self, and amplification of effector responses leads to tissue damage, release of new autoantigens, and an amplified and on-going response. This proceeds unless the autoantigen can be completely destroyed, as occurs in autoimmune thyroiditis. This scenario would be consistent with the fruitless search for infectious origins of autoimmune diseases.

Overall, autoimmunity is one of the most common causes of disease, and in the absence of therapeutic intervention, autoimmune diseases become chronic with a high level of morbidity. Treatment is aimed at reducing symptoms, preventing progressive damage and dampening inflammation, and if the disease terminates in loss of autoantigen, treatment is by replacement therapy, such as thyroid hormone replacement in autoimmune thyroiditis, and insulin replacement in type 1 diabetes. New generation drugs being developed for treatment are expensive, not without side-effects, and often employed as drugs of last resort rather than as therapies rationally selected by an understanding of the disease process. Knowledge of the ways in which autoantibodies cause damage, and the biochemical and immunological pathways that are involved may identify the particular clones of B-cells responsible for the most damaging or distressing symptoms, and selection of appropriate treatments to suppress or eradicate them.

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Author Contributions

Merrill Rowley wrote the manuscript and prepared the Tables. Senga Whittingham provided detailed clinical input and reviewed and revised the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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