Remnant Epitopes Generating Autoimmunity: From Model to Useful Paradigm

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Autoimmune diseases are defined as pathologies of adaptive immunity by the presence of autoantibodies or MHC-restricted autoantigen-reactive T cells. Because autoreactivity is a normal process based on mechanisms producing repertoires of antibodies and T cell receptors, crucial questions about disease mechanisms and key steps for interference have been outstanding. We defined 25 years ago the ‘remnant epitopes generate autoimmunity’ (REGA)-model in which extracellular proteases from innate immune cells generate autoantigens. Here, we refine the REGA-model, tested in diseases ranging from organ-specific autoimmune diseases to systemic lupus erythematosus. It now constitutes a paradigm in which remnant epitopes generate, maintain, and regulate autoimmunity; are dependent on genetic and epigenetic influences; are produced in a disease phase-specific manner; and have therapeutic implications when targeted.

Autoimmunity: What Was New 25 Years Ago?

The definition of autoimmune diseases needs to be clear, because progress in diagnosis, prognosis, and therapy is required. We adhere to the original finding that the presence of pathogenic antibodies and, by extension, autoreactive T cells against proven host or ‘self’ molecules is mandatory. This definition implies disease symptoms and proof of pathogenic autoreactive antibodies or T cells against a defined self-antigen for a considerable period. Autoimmune diseases are chronic and do not heal (Box 1). In times when certain cancers might become curable, this may sound like a shocking, pessimistic view. However, similar to cancer and other diseases, prevention is an essential element against autoimmune diseases. This knowledge incites a quest for etiological factors. The second element embodies a critical attitude toward basic, preclinical, and clinical findings. New treatments are ‘discovered’ serendipitously and recent insights have overturned past therapeutic approaches. More engagement can improve care for autoimmune disease patients.

Because the antibody and T cell repertoires arise on a purely genetic basis, every host has the intrinsic propensity to develop autoimmune diseases. Consequently, questions arise regarding how autoimmune diseases are initiated and perpetuated and which factors contribute to explain clinical observations, such as: (i) gender and population differences in incidence and prevalence; (ii) associations of autoimmunity with genetics and (iii) with environmental factors; and (iv) autoimmunity linked to immunodeficiencies. Immunodeficiencies are classically defined as either primary, depending on a genetic basis, or secondary, when the disease is acquired (e.g., by immunosuppressive therapy). This semantic dichotomy of primary and secondary needs to be avoided for autoimmunity, because both components of genetics (= host) and environment (= epigenetics) are always present (*vide infra*). Our hypothesis is that cells and molecules of the innate immune system, rather than lymphocytes and their molecules, start autoimmune reactions by cytokine-regulated proteolysis yielding remnant epitopes. We provide an update of the evidence for our hypothesis, dubbed ‘**remnant epitopes generate autoimmunity**’ (REGA)

Highlights

Every autoimmune disease needs to be defined on the basis of autoantigens yielding reactivity with specific T cell receptors or antibodies. A clear distinction with autoinflammation is possible.

Extracellular proteolysis of host molecules (as a post-translational modification) is one of several, but important, innate immune mechanisms in the generation, maintenance, and regulation of autoimmunity. It leads to so-called remnant epitopes and it anticipates intracellular proteasomal and lysosomal proteolysis for MHC-I- and MHC-II-specific antigen presentation.

Cytokine-regulated extracellular proteolysis and other post-translational modifications of autoantigens may be executed by host enzymes, including complement molecules, matrix metalloproteinases, and deiminases, or by microbial enzymes. This knowledge implies: (i) a more prominent role for innate immune molecules and cells in autoimmune diseases; and (ii) a stronger link of these protein modifications with infections.

By better defining genetic and immunological biomarkers associated with autoimmune diseases, it may be possible to characterize susceptible individuals. This may be useful not only for diagnosis and prognosis but also for prevention.

To develop future therapies against various autoimmune diseases, more studies are needed to define beneficial and detrimental environmental factors (e.g., nutrition, microorganisms). Present-day successful approaches with the use of innate immune modulators, such as interferon (IFN), anti-TNF (tumor necrosis factor) and tetracyclines, need to be complemented with therapies based on environmental factors, e.g. nutrition and anti-microbial agents.

Box 1. Criteria for Definition of Autoimmune Diseases

Not all chronic conditions with an inflammatory component may be named autoimmune diseases (Table I). Presently, the technologies for diagnosis have evolved sufficiently to include as an absolute criterion a readout with proof of antibodies or T cell reactivity against a host (self) antigen. Genetic and epigenetic factors are both present but their relative weights may vary; hence, their inclusion as a 'relative criterion'. With these criteria, some diseases discussed in this opinion article may fall short and should be named 'probably autoimmune', whereas rare forms of Parkinson's disease may now revert under the strict definition of autoimmune diseases.

Table I. Criteria for Definition of Autoimmune Diseases

Criterion	Presence of criterion		Detection method
	Absolute	Relative	
Proven host molecule as autoantigen	Antibodies or T cells		Antibody titer or epitope scanning
Inflammation	Present		Various (e.g., fever, cytokines, acute phase reaction, altered glycosylation)
Chronic condition	Present		Disease for >1 month or repetitive exacerbations
Host genetics		Variable	DNA and HLA analysis
Epigenetics/environment		Variable	Histone modification, infection

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(see [Glossary](#)), for common autoimmune diseases, then illustrate novel insights from uncommon, systemic, and 'new' autoimmune disorders, and end with opinions on how to use this information for better diagnosis, prognosis, and therapy.

The REGA Model of Autoimmunity

Around 1995, autoimmunity research was T cell centered [1–4]. The role of B lymphocytes and antibodies was already well established. On one side, a prototypical organ-specific autoimmune disease, such as **Hashimoto's disease**, was explained in terms of pathogenesis by the production of autoantibodies against **thyroglobulin**. In this condition, antibodies bind to a tissue-specific self-antigen and the immune complexes activate the **complement** system and induce inflammation, cytotoxicity, collateral damage, and gradual tissue destruction, causing hypothyroidism [5]. On the other side of the spectrum – from organ-specific to generalized autoimmune diseases – sera from patients with **systemic lupus erythematosus (SLE)** react with antigens present in all tissues. The observation of **antinuclear antibodies (ANAs)** and antibodies against ubiquitous cytoskeletal proteins hinted at an explanation for why such patients might develop multiorgan involvement, thus requiring general immunosuppression [6].

Twenty-five years ago, knowledge of MHC restriction [7] and the role of T cells in viral infections and autoimmune diseases incited in-depth structural studies of how peptides are presented in MHC and of the T cell receptor (TCR) subunits [8–10]. By transfer of myelin peptide-specific T cell clones in mice, it was possible to induce **experimental autoimmune encephalomyelitis (EAE)**, an animal model of multiple sclerosis (MS) [11]. These studies regarding MS stood out, and the readout of **myelin basic protein (MBP)** peptide presentation by MHC class I or II to activate CD8⁺ or CD4⁺ T cells [12] reinforced the concept that T cells are essential and that approaches for therapy of autoimmune diseases might come from this front.

By 1995, the **Rega Institute** had become a powerhouse in antiviral, cytokine, and protease research due to the establishment of a semi-industrial plant for mammalian cell culture for the production of natural human glycoproteins and screening of antivirals. Here, the first batches of human fibroblast-derived interferon- β (IFN- β) [13] and melanoma-cell-secreted tissue-type

plasminogen activator (t-PA) [14] were produced for clinical testing. IFN- β remains a treatment option for patients with relapsing–remitting (RR) MS [15] and t-PA is used for thrombolysis [16]. During the period 1985–1995, the foundations of new insights into autoimmunity were established by discoveries of human cytokines [17,18], chemokines [19,20], and the protease gelatinase B/**matrix metalloproteinase (MMP)**-9 [21]. Concentrations of this latter cytokine-regulated protease were used as a readout for certain autoimmune diseases, including rheumatoid arthritis (RA) [22] and MS [23]. Integration of knowledge culminated in the concept of ‘remnant epitopes generate autoimmunity’, since then named the REGA model [24]. Four elements were innovative. First, the spotlight was focused on myeloid cells rather than on lymphocytes. Second, a switch was made in terms of molecules: from antibodies and TCRs to cytokines and proteases as innate molecules. Third, proteolysis within the framework of autoimmune reactions was refocused in time and space from later intracellular (proteasomal and lysosomal) to prior extracellular events. Fourth, the integrated view provided explanations for the selection from genetically determined repertoires of antibodies and TCRs by priming/boosting events for the initiation and maintenance of autoimmunity. With the recent introduction of techniques for single-cell transcriptomics and protein expression analysis, it has become possible to determine the immune cells and molecules that are present at various stages of autoimmune disease development [25–28]. The data from these studies corroborate the REGA model and stimulate research to discover the autoimmune nature of specific diseases.

Present State: From MS and RA to Diabetes

Common autoimmune diseases are generally characterized by basic clinical and pathological features combining genetic and epigenetic elements (Box 2).

MS

The disease progression in MS comes in two major variants: fluctuating RR MS and primary progressive (PP) MS, both having lesions at various stages (Figure 1). It has remained a chicken-or-egg debate whether the initiation of MS occurs via sentinel T cells or myeloid cells. The presence and activation of macrophages and **microglia**, prototypic myeloid cells [38], is a common observation in early MS lesions [39]. In MS patients and mice with EAE, leukocytes accumulate between the endothelial and parenchymal basement membrane layers of the **blood–brain barrier (BBB)** [40], histopathologically described as **vascular cuffing**. Providing

Box 2. Basic Elements of Autoimmune Diseases

Autoimmune diseases occur in phases, are based on genetics and epigenetics, and have a major involvement of innate immune mechanisms. Autoimmune diseases are dynamic entities with ups (relapses) and downs (remissions). The simplest way to explain this is by reflecting on RR MS and bouts of RA. Often, the first clinical sign in MS patients is a temporary vision loss, named optic neuritis, or paralysis, whereas RA patients develop transient and painful joint inflammation. Reminiscent of a classical antibody response, relapses are characterized by four phases: lag, exponential, plateau, and decline (see Figure 1 in main text and *vide infra*). However, in other common autoimmune diseases, including diabetes and SLE, these phases may smolder invisibly and disease may rage for years, causing considerable damage before a physician is consulted or the correct diagnosis is made. Hence, the definition of diagnostic and prognostic biomarkers for early disease detection and preventive measures is essential.

The lists of genetic susceptibility factors are increasing for every autoimmune disease. However, the MHC or human leukocyte antigen (*HLA*) locus remains in pole position [29]. Besides *HLA*, other important genes relevant for autoimmune diseases encode critical transcription factors such as **autoimmune regulator (AIRE)** [30] and FoxP3 [31]. Recently, miRNAs [32] and long noncoding RNAs [33] have been studied as on/off switches of immune response genes at the interface between genetics and environmental influences/epigenetics, besides serum glycan biomarkers [34] and marker combinations [35,36].

Because MHC molecules are mainly expressed on antigen-presenting myeloid cells and their expression is enhanced by proinflammatory cytokines in all infections, the critical link of innate immunity with autoimmune diseases can be reinforced by the REGA model. Some MHC molecules are shared or complemented [29,37] in multiple autoimmune diseases.

Glossary

α -Synuclein: abundant cytosolic protein in brain cells with a presumed function in synaptic vesicle traffic. Aggregation observed in autopsy samples in Parkinson’s, Alzheimer’s, and other neuropathies.

Achalasia: swallowing disorder of the esophagus. Patients have autoantibodies or T cell reactivities against various proteins of the myenteric plexus.

Antinuclear antibodies (ANAs): directed against common nuclear macromolecules, including nucleic acids and molecular complexes of the splicing apparatus; can induce systemic autoimmunity.

Autoimmune regulator (AIRE): transcription factor produced by antigen-presenting cells in the medulla of the thymus; regulates ectopic expression of host proteins to assist in tolerance induction.

Axonopathy: damage to neuronal axons.

Blood–brain barrier (BBB): histological structure with two basement membranes in CNS blood vessels, limiting leukocyte entry. One is produced by endothelial cells, one is made by astrocytes.

Complement: tuning and amplification system of more than 20 molecules of innate host defense with cytotoxic actions against microbes and host cells; often transmits recognition by antibodies.

Demyelination: disintegration of the myelin sheet around neuronal axons; slows electrical signal transduction; clinically observed as vision loss and striated muscle paralysis.

Epitope scanning: determination of autoantigenic segments of (glyco) proteins measuring interactions with host antibodies or subsets of reactive T lymphocytes.

Experimental autoimmune encephalomyelitis (EAE): various forms of animal models reflecting clinical elements of MS, mainly muscle paralysis.

Granulomatous anterior uveitis: painful eye inflammation of the anterior chamber and iris. The most common causes are trauma and infections; can accompany autoimmune diseases.

Hashimoto’s disease: autoimmune disease of the thyroid gland. Sera from such patients contain immunoglobulins that react with thyroglobulin, the major carrier protein of thyroid hormones.

Figure 1. Dual Roles of Single Proteases on Remnant Epitopes in Autoimmunity. Relapsing–remitting multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE) exacerbations (y-axis at right) present clinically in four phases named latency, relapse, plateau, and remission. These are reminiscent of the lag, log, plateau, and decline phases in classical seroconversion after vaccination (y-axis at left). The latent onset corresponds to the proteolytic generation of remnant epitopes, activating autoreactive T cells and inducing antibodies and immune complexes [e.g., against myelin basic protein (MBP)]. Deletions of the *Fas* and *Mmp9* genes protect mice against the onset of EAE development. Once EAE is established, the remission of the disease is delayed in the absence of matrix metalloproteinase-9 (MMP-9). MMP-9 degrades immune complexes containing MBP, not by cleaving the immunoglobulins but by cleaving the MBP antigen [76]. Consequently, MMP-9 generates remnant epitopes of MBP in the initiation phase of EAE and thus contributes to the exacerbation of the disease. However, it plays a protective role in the remission phase by cleaving antigens out of the immune complexes [76]. Similar elements are essential in systemic lupus erythematosus (SLE), where MMP-9 is also involved in the clearance of immune complexes [75]. MMP-9 has been studied extensively in autoimmune diseases. However, other proteases, including serine proteases of the complement system and tissue plasminogen activator (t-PA), may possess similar functions. For instance, compared with wild-type animals, *Tpa*^{-/-} mice have delayed-onset EAE, increased severity, and delayed recovery from EAE [93]. The illustration shows an antibody response, but similar phases and timings may be applied to T cell reactivity against MHC-presented autoantigens. Another aspect, not illustrated here, is the action of additional enzymes for post-translational modification of autoantigens [94].

these leukocytes remain contained between the two basement membranes, no neurological symptoms are evident [41]. **Demyelination** and loss of axons (**axonopathy**) and neurons (**neuronopathy**) are other hallmarks of MS and EAE lesions. Whereas it remains unclear what is cause or consequence, cytokines and regulated proteases [24] as ‘disease readouts’ have become more prominent and perfected the REGA concept [42]. This generally led to successful Phase III studies (e.g., with minocycline as an inhibitor of metalloproteinases and as a drug effective against MS) and to recommendations for prevention by better hygiene against infections coupled to nutritional recommendations (*vide infra*).

It was of intellectual importance to provide examples of remnant epitopes. This was possible with the development of **epitope scanning**, a technique originally used with peptides from water-soluble MBP and, later, with citrullinated peptides [43]. Another example was alpha B-crystallin, a molecule previously defined as a critical autoantigen in MS and EAE [44]. Rather than claiming it as a detrimental cause of MS, we suggested that intact alpha B-crystallin is a protective

Matrix metalloproteinases (MMPs): a family of mammalian proteases, besides the serine, cysteine (or thiol), threonine, and aspartate proteases; contain a zinc ion; involved in extracellular matrix degradation and the regulation of immune reactions by, for example, processing of cytokines and chemokines.

Microglia: myeloid cells derived from hematogenic precursors; involved in MS pathology as antigen-presenting cells.

Myelin basic protein (MBP): major water-soluble myelin protein. Its peptides are recognized by antibodies and T cell receptors in MS and EAE.

Myenteric plexus: grid for the muscles of the intestine; network of neurons supporting the innervation of smooth muscles for the control of intestinal contractions.

Neuronopathy: damage observed in neurons caused by extrinsic toxins and neurotropic viruses or by intrinsic molecular aggregations within neurons, such as tau protein in Alzheimer’s disease.

Poliosis: depigmentation of eyelashes.

Proteofoms: how proteins are presented in biological systems in relation to protein structure and post-translational modifications, including proteolysis, glycation, and citrullination.

Rega Institute: founded in 1954 at the University of Leuven, Belgium for the study of infections and immunity; named after Henri-Joseph Rega (1690–1754), philanthropist and university rector.

Remnant epitopes generate autoimmunity (REGA): immunology paradigm addressing the involvement of myeloid cells and innate immune enzymes at the onset, maintenance, and resolution of autoimmune diseases.

Stiff-person syndrome: rare autoimmune disease with antibodies against glutamic acid decarboxylase. Patients develop painful stiffness of the back muscles and gait problems.

Sunset-glow fundus: observed in VKH disease; results from vitiligo or depigmentation in the choroid. The absence of melanin results in an orange-red coloration of the eye fundus.

Systemic lupus erythematosus (SLE): multiorgan or systemic autoimmune disease with antibodies against many different intracellular or intranuclear autoantigens.

Thyroglobulin: major protein in the thyroid gland colloid from which the thyroid hormones are generated; one of the first-defined human autoantigens.

molecule in EAE, whereas its remnant epitopes after extracellular proteolysis cause/enhance EAE and MS symptoms/outcomes [45]. When alpha B-crystallin knockout mice (*Cryab*^{-/-}) were generated, these animals developed worse EAE symptoms and more neuroinflammation than wild-type mice on a similar genetic background [46]. Hence, the intact alpha B-crystallin molecule was confirmed as a protective factor, whereas the remnant epitopes enhance autoimmunity and were valued as pathogenic determinants [47].

The demonstration of proliferation of T cell clones from various rat strains after stimulation with peptides from MBP [11,48] incited the study of adaptive immune therapies. The hope was to eliminate specific T cells recognizing autoantigens. Paradoxically, success in defining treatments for MS did not come with the use of any specific autoantigen, or with the elimination of autoantigen-specific T cell clones, but instead with the development of the drug glatiramer acetate, a peptide mixture based on the composition (and not the sequence) of MBP. Although multiple mechanisms of action have been proposed for the function of glatiramer acetate [49], it is accepted that these peptide mixtures interfere with presented remnant epitopes of MBP and block the activation of various autoantigen-specific T cell receptors. Like IFN- β [15], injected subcutaneously or intramuscularly in large cohorts of MS patients glatiramer acetate needs to be injected subcutaneously and has achieved only a slowing of MS progression [49]. Notwithstanding this, these two drugs have reinforced the concept that MS might be treatable through the modulation of innate immune mechanisms, because endogenous IFN- β is mainly produced by fibroblasts and glatiramer acetate peptide mixtures are not specific.

RA

Collagens, in particular collagen type II, are important components of cartilage and bone, and remnant epitopes represent autoantigens in RA. Due to extensive post-translational modifications of collagen/gelatin and its remnant epitopes and those of other autoantigens, studies in RA were lagging behind investigations of MS. Triple-helical collagen is crosslinked and glycosylated and may become citrullinated and glycated, making this structure quite resistant to proteases [42]. Joint collagen is destabilized after a single cut by one of the three collagenases belonging to the MMPs. The generated gelatin becomes prone to further cleavages by gelatinase A/MMP-2 and B/MMP-9 [50]. With the use of epitope scanning experiments, human MMP-9 was demonstrated to cleave bovine collagen into pieces that activate the proliferation of human T lymphocytes in an antigen-specific way; this is in support of the REGA concept in RA [50–52]. It is important to add that, besides MMPs, other proteases, including serine proteases of the complement system, may generate remnant epitopes of collagen [24,42,53]. From another angle, inflammation with neutrophils, as an innate immune mechanism, is an accepted hallmark in early (autoimmune) arthritis lesions [54,55]. Thus, in RA, cells and molecules of innate immunity may also contribute to the pathogenesis and might constitute better therapeutic targets than those in adaptive immune mechanisms; however, this remains to be further assessed.

Type 2 Diabetes

As technologies progressed, remnant epitope generation, scanning analysis, and cellular immunophenotyping were performed in diabetes. In type 2 diabetes, constant low-grade inflammation is maintained by the activation of the receptor for advanced glycation end products (RAGE) on myeloid cells [42]. This induces proteases and the propensity to cleave insulin into remnant epitopes. In a mouse model of streptozotocin-induced diabetes, acute pancreatic inflammation yielded extracellular proteolysis of insulin into remnant epitopes and autoantigenic stimulation relative to control animals [56,57]. The implications are: (i) that in susceptible hosts, any form of pancreatitis might enhance activation of autoreactive T lymphocytes; and (ii) tight glycemia control and reducing inflammation may have an impact on curbing diabetes.

Vascular cuffing: accumulation of white blood cells between the endothelial and astrocytic layers of the basement membranes CNS, as observed in biopsies on autopsy.

Vitiligo: sign of a medical condition, characterized by depigmentation of the skin. It is caused by the patchy destruction or loss of melanin-producing cells in the skin (melanocytes).

Vogt-Koyanagi-Harada (VKH)

disease: autoimmune disease against tyrosinase or tyrosinase-related proteins and destroying melanocytes in skin, the uveal tract of the eyes, and the inner ear.

Table 1. Remnant Epitopes and Post-translational Modifications in Human Autoimmune Diseases and Mouse Models^a

Disease	Autoantigen	RE	T cell reaction	Antibody	PTM	Refs
MS	Myelin proteins	✓	✓	✓	Citrullination	[12,43]
RA	Collagens	✓	✓	✓	Glycosylation	[50,51]
	Several proteins				Citrullination	[54]
Type 2 diabetes	Insulin, GAD65 GRP78	✓	✓	✓	Glycation Citrullination	[42,63] [60,61]
SLE	Intracellular macromolecules	✓	✓	✓	Probable	[72,74,75]
VKH	TRP-1, tyrosinase	✓	✓	✓	Unknown	[64]
Mouse model						
EAE	Myelin proteins	✓	✓	✓	Citrullination	[43,76]
CIA	Collagen	✓	✓	✓	Glycosylation	[50,51]
AIA	Collagen	✓	✓	✓	Unknown	[53]
Diabetes	Various	✓	✓	✓	Unknown	[56,57]
SLE	Various	✓	✓	✓	Probable	[74,75]

^aAbbreviations: AIA, antibody-induced arthritis; CIA, collagen-induced arthritis;; PTM, post-translational modification; RE, remnant epitope; TRP-1, tyrosinase-related protein-1; ✓ denotes presence.

Besides insulin, glutamic acid decarboxylase (GAD65) and, recently, glucose-regulated protein 78 (GRP78) were defined as autoantigens in human diabetes and mouse models (Table 1). Furthermore, for these autoantigens, not only proteolysis into remnant epitopes but also other

Box 3. Remnant Epitopes in SLE

SLE is an autoimmune disease characterized by antibodies against nuclear and abundant cytoplasmic antigens. Common nuclear antigens are DNA, RNA, and molecules of the splicing machinery, whereas actins and tubulins are prototypic cytoplasmic SLE autoantigens. More than 100 different autoantibodies have been defined in SLE [70]. Related to the REGA model, it was unclear how extracellular proteases might access these intracellular substrates [72]. A second question was what are the clinical effects of proteases and protease inhibitors according to disease phases; these emerged in recent studies where MMP-9 was found to play a beneficial resolving role *in vivo* in a mouse model of SLE [74,75].

First, when cells die, ubiquitous intracellular proteins (and DNA and RNA) enter the extracellular milieu in an inflammatory environment. Remnant epitopes (e.g., of intracellular actins and tubulins) are generated by inflammatory proteases and may be further enzymatically modified into neopeptides and activate autoreactive CD8⁺ cytotoxic T lymphocytes and CD4⁺ T cells, assisting autoantibody-producing B lymphocytes. In doing so, they further boost the host immune system enhancing autoimmune reactions. The efficient cleavage and elimination of abundant actins (entering the extracellular milieu) may serve an immediate protective function, as extracellular and intravascular actin is highly thrombogenic [73] and leads to diffuse intravascular coagulation (DIC). However, this potentially life-saving action by proteases comes at a cost, as the remnant epitopes of the proteolysis in host DIC may boost autoantibody production; hence, the gradual progress of disease parameters in SLE.

C57BL6^{pr/pr}, also named B6^{pr/pr}, mice with deletion of the Fas protein develop a SLE-like syndrome with splenomegaly, lymphadenopathy, and autoantibody formation. When Lpr animals are crossed with *Mmp9*^{-/-} mice on the same genetic background, the disease is significantly exacerbated, providing convincing evidence for a protective role of MMP-9 in autoantigen clearance [74]. This phenomenon aligns with the REGA paradigm: (i) in the absence of (intact) autoantigen, no remnant epitopes are generated; and (ii) autoantigens may be cleared by MMP-9, even when captured in immune complexes [75]. In this sense, the action of proteases in the mouse SLE model is phase-specific, as is the case with the EAE mouse model for MS in humans [76]. In these studies [75,76], it came as a surprise that IgM (900 kDa) and IgG (150 kDa) were not cleaved, whereas much smaller EAE or SLE autoantigens, such as MBP and actin, respectively (monomers are about 20 kDa), were efficiently cleaved by MMP-9 into remnant epitopes even when the latter were captured into immune complexes [75,76]. The presumed phase-dependent dual role of MMP-9 suggests that, in the onset of autoimmune stimulation, the protease network may be detrimental and needs to be ‘shut off’, whereas in the later phases, once immune complexes aggravate the pathology, proteolysis may be beneficial for their elimination. For these reasons, we caution against blunt use of MMP inhibitors for the treatment of systemic and immune-complex-mediated autoimmune diseases [75].

post-translational modifications, including citrullination by protein arginine deiminases (PADs), might contribute to 'auto'-immunogenicity [42]. Again, the link to myeloid cell biology, rather than to lymphocytes, may be straightforward. In patients with type 1 or 2 diabetes, for example, neutrophils carrying RAGE are thus triggered by glycated proteins to release proteases, which generate remnant epitopes [58]. Because neutrophils in noninfectious diseases [59] also produce PAD enzymes [60], citrullinated remnant epitopes have been suggested to potentially be relevant neoantigens, probably for both type 1 and type 2 diabetes [61]. Consequently, in forms of diabetes, (i) proteases can generate autoantigens, which (ii) may then become chemically modified into neoantigens (e.g., by glycation and citrullination).

Novel Insights from Uncommon Autoimmune Diseases

A hallmark of untreated autoimmune diseases is progression: every time remnant epitopes are generated, these can enhance T or B cell reactivity (Figure 1) [42]. Such exacerbations increase organ damage, resulting in gradual loss of function. Besides the prevention of autoimmunization

Key Figure

Remnant Epitopes and the Interplay between Host and Environment

Figure 2. Intact autoantigenic proteins are cleaved into multiple remnant peptides by host-derived or environmental enzymes belonging to various protease classes [24,42]. Additional enzymes (e.g., glycanases, deiminases) assist to broaden qualitatively the remnant epitope repertoire and the proteoforms of the intact autoantigens (not shown). These actions may occur in the extracellular milieu with enzymes from myeloid cells, such as neutrophils and macrophages, complement proteases, and plasmin, and they anticipate and complement the intracellular proteolytic actions of antigen-presenting cells (APCs) and stimulation of the adaptive arms of the immune system. Interference (in red) may be at the level of the host or the environment. For instance, host-derived matrix metalloproteinases may be inhibited by small-molecule drugs [90,91] and by monoclonal antibodies when high specificity is required [92]. As an example related to environmental influences, the bacterial microbiome may be altered by diet [88] and by antibiotics. Some antibiotics, such as minocycline, may be endowed with double functions, because these inhibit bacteria and hence lower the load of environmental enzymes while also inhibiting host metalloproteinases [78–80,88]. Abbreviations: BCR, B cell receptor; PTM, post-translational modification; TCR, T cell receptor.

(see above), the resolution of an established autoimmune disease comprises (i) prevention of restimulation by autoantigen(s); and (ii) elimination or reduction of present autoantibodies or T effector cells. The long-sought key to the latter tolerance induction has not yet been found. Common diseases such as diabetes, MS, and SLE evolve into life-threatening conditions because of the involvement of multiple or irreplaceable organs. For less common autoimmune diseases, research investments are scarce. Nevertheless, their study yields useful information and lessons to apply to common diseases. We illustrate this with examples of autoimmune diseases of the esophagus and eyes.

In **achalasia**, the neuronal system that signals relaxation of the esophageal muscles, called the **myenteric plexus**, is destroyed. First, autoantibodies and various subsets of autoreactive T cells have been detected against plexus-specific molecules, including amphiphysin and paraneoplastic antigen MA2 (PNMA2) [62]. By analyzing serum concentrations, MMP-2 and MMP-9 **proteoforms** became novel parameters with potential use in the diagnosis and prognosis of achalasia subtypes [63]. Second, the presence of these enzymes *in situ* in affected esophageal tissues and autoantigen cleavage studies resulted in the discovery of remnant epitopes in achalasia. This implied that MMP inhibition might constitute a new putative therapy for achalasia [63].

Vogt–Koyanagi–Harada (VKH) disease is an ophthalmological autoimmune disease with **vitiligo**, **poliosis**, and eye involvement, leading to blindness and other neurological symptoms [64–66]. Innate immune mediators, including the cytokine interleukin (IL)-10 and the chemokine CXCL13, are increased in the aqueous humor fluid of VKH patients versus disease controls [67]. However, it is more relevant to mention that, in VKH disease, excellent progress has been made on the therapeutic front. The addition of immunosuppressive mycophenolate mofetil to corticosteroids as first-line therapy in patients with initial-onset acute VKH disease prevents progression of the disease to chronic recurrent **granulomatous anterior uveitis**, the development of **sunset-glow fundus** and late complications. Furthermore, patients can discontinue treatment without relapse of inflammation [66,68]. These findings suggest that catching the therapeutic window of opportunity in initial-onset acute uveitis associated with VKH disease and achieving early remission can change the disease outcome completely. Thus, early diagnosis and proper immunosuppressive therapy are crucial steps toward achieving optimal control of uveitis associated with VKH disease. This window represents the early disease phase, likely because the underlying disease process is not fully matured [69] (Figure 1). The dual treatment stops the primary autoimmune reaction in such a way that most patients are cured [69]. This example represents a long-sought objective in the field of autoimmunity; namely, a ‘cure’ without need of further treatment. It points to the importance of: (i) early diagnosis by first disease signs; and (ii) avoiding remnant epitope generation by aggressive therapy.

The growing conviction is that, once the autoimmune process is initiated, aggressive immunosuppressive treatments are preferred above soft ones, as is also clear from progress in clinical rheumatology. It is our opinion that similar approaches are testable for other autoimmune diseases, in particular now that disease susceptibilities may be predicted. In conclusion, rare autoimmune diseases such as achalasia and VKH disease constitute reality checks for the REGA paradigm in terms of diagnosis, prognosis, and even therapy.

Recent Additions: From Organ-Specific Autoimmunity to Systemic Autoimmunity

Compared with MS, RA, achalasia, and VKH disease, testing the REGA model has been more difficult in the study of SLE, in which the repertoires of autoantigens are broad [70] and include

Clinician's Corner

The definition of autoimmune diseases needs to be evidence-based and a diagnosis must be based on clinical assessment and demonstration of the presence of reactivity against host antigens by either defined autoantibodies or autoreactive T cells. Present-day high-end genetic analysis not only provides information about disease susceptibilities but also strengthens the REGA concept that myeloid cells and their products are key players at disease onset and resolution [83,84].

Forty years ago, RA treatment was performed by gradual switching from anti-inflammatory drugs [aspirin, non-steroidal anti-inflammatory drugs (NSAIDs)] over corticosteroids to immunosuppressive drugs, such as methotrexate. By contrast, aggressive therapy is presently advocated from the disease onset onwards [85]. Antigen-specific treatment has not yet hit the market, whereas interference with innate immune molecules (e.g., cytokine and chemokine signaling blockade, protease inhibition) is now in daily practice [85–87].

Aggressive treatment at disease onset with immunosuppressive drugs, which can also target myeloid cells, has led to the cure of a previously untreatable autoimmune disease, VKH (autoimmunity affecting melanocytes, including those of the uveal tract) [64]. It used to be an irreversible blinding disease. With a mycophenolate mofetil and prednisolone combination at first presentation, the production of remnant epitopes is potentially controlled; thus, autoimmune activation may not occur or is at least postponed. Some of these patients are presently off medication, whereas they would be on chronic therapy otherwise without any guarantee of avoiding severe side-effects, including blindness [68,69].

Assessment of precise cofactors of common autoimmune diseases – including diabetes and MS – will be instrumental in better prevention of autoimmune pathologies. Once the autoimmune process is initiated, therapeutic measures to reduce infections and all stimuli of inflammation (hyperglycemia leading to advanced glycation end products, traumata,

molecules that do not fit the classical models of MHC-based activation of T cells (e.g., DNA, RNA). However, degradomics and mouse genetic studies helped to solve some aspects of this issue. Degradomics technologies are intended to define all of the substrates of single proteases [71]. This aspect was studied for MMP-9 substrates in dying or dead cells. Other proteases (e.g., complement proteases, caspases) may be studied equally well in this context. Many intracellular proteins, such as actins or tubulins, are efficient substrates of MMP-9, yielding remnant epitopes [72]. How their presence may contribute to the initiation and resolution of SLE is explained in Box 3 [73–76].

From Host-Derived Proteases to Enzymes of Microbial Origin

Conceptually, once an autoimmune disease has started, immune memory will be boosted each time the autoantigens reappear. This implies two crucial elements for therapy: every autoantigen presentation must be avoided or minimized and treatments to stop such ‘immunizations’ need to be immediate and potent. For RA this has become common knowledge. Around 1980, RA treatment was started softly with anti-inflammatory drugs to keep the ‘stronger’ medications for later stages. This approach is now completely abandoned. Today, blockade of tumor necrosis factor (TNF) activity and other RA treatments represent strong interference with immune processes [85–87].

Remnant epitopes are the result of proteolysis with qualitative and quantitative aspects. Where do the proteases cleave? How many remnant epitopes are generated? These two questions are important elements, but do not represent all aspects of autoantigenic neoepitopes. Additional modifications are possible (e.g., citrullination) (Table 1). As reviewed elsewhere [42], both proteolysis and chemical or enzymatic modifications may be executed by the host, but also by microbial molecules. Citrullination by bacteria was elegantly exemplified recently [77] and places environmental aspects in the spotlight (Figure 2, Key Figure).

New Spin-Offs: From Stiff-Person Disease to Specification of Parkinson’s Disease

The most common form of RR MS is becoming a better-treatable disease in line with the REGA paradigm, by protease inhibition and by repurposing of known drugs. On the basis of structure–function studies of tetracyclines as inhibitors of MMPs [78] and translational studies in mice [79], minocycline is now a notable example for the treatment of MS [80].

Similar challenges are taken up for nonclassical autoimmune reactions in the central nervous system (CNS). Insights have been obtained about autoimmune epilepsy [81]. In this condition, an important distinction has been made between secreted, membrane-bound (e.g., receptors for NMDA, AMPA, and GABA_B), and intracellular (e.g., GAD65) autoantigens [81]. The latter example is interesting in that it reinforces our original point about autoimmune disease definition; namely, the requirement of proven autoantigens and B or T cell reactivity (Box 1). **Stiff-person syndrome** is defined as an autoimmune disease on the basis of (proven) autoantibodies against GAD65. This disorder often mimics the clinical presentation of MS forms (for which the autoantigens may be not proven) [82]. Serendipitously, GAD65 was found to be cleaved into remnant epitopes by MMP-9 [63], yielding the possibility that MMP-9 inhibition might be beneficial for treatment of this condition.

Finally, recent studies suggest that peptides from **α-synuclein** can lead to helper and cytotoxic T cell reactivity, with a view that this may be causative for Parkinson’s disease [25]. How these autoantigens are generated *in vivo* has not been investigated and the authors suggest endogenous cleavage by serine proteases (e.g., plasmin, chymotrypsin). However, other inflammatory

etc.) need to be implemented. Nutrition and its dramatic effects on gut microbiota are presently scrutinized as cofactors for many autoimmune diseases [88]. Therefore, anti-inflammatory drugs and protease inhibitor medication, such as the antibiotic minocycline, may be beneficial [80].

Another clinical consequence is related to disease phases. The initiation of autoimmune processes may be compared with primary immunization after vaccination (see Figure 1 in main text). Exacerbations of MS and RA often coincide with infections, and these conditions may be compared with boosting after secondary vaccination. Resolution of an active phase of autoimmune disease may occur when antibody titers and T cell numbers fade out. Because proteases play different roles in individual disease phases [75,76,89], therapeutic approaches with protease inhibitors need to be phase-specific. Fortunately, novel [90] and repurposed [91] small compounds as well as highly specific monoclonal antibodies [92] exist for future clinical testing.

Early diagnosis of any autoimmune disease and the eventual detection of susceptibility are key prevention measures. We contemplate that any autoimmune boost severs the autoimmune process and the clinical condition. Therefore, the present-day search for good diagnostic and prognostic biomarkers and parameters to predict therapy outcome is paramount.

Of note, autoinflammatory diseases are immunodeficiencies and not autoimmune diseases, as the former do not involve defined autoantigens. Also, patients with an autoinflammatory disease may develop autoimmune diseases when reactivity to at least one defined autoantigen is established.

extracellular proteases may be involved (*vide supra*). Regardless, the data fit the REGA paradigm and are in line with all of the proposed criteria to define such – maybe rare – forms of Parkinson's disease as genuine autoimmune diseases. This may open the way to anti-inflammatory treatments for patients with these proven autoantigens. In addition, with refined methodologies at the single-cell level, recent studies have illustrated other aspects of the REGA paradigm; namely, that cells and molecules of innate immunity may execute memory functions in neuroinflammation [26] and represent early players in EAE and MS [27,28].

Concluding Remarks

The presence of autoantibodies and autoreactive T cells defines autoimmunity. If we envision that their presence is not the cause but the result of other processes, one might end with the REGA paradigm of innate immune activation as a trigger of autoimmune processes (see [Outstanding Questions](#)). This overturn of cause and consequence for B and T lymphocytes, 25 years ago, has led to a better understanding of the pathogenesis of a broad spectrum of autoimmune diseases. It also fits with optimized and more efficient therapies, including aggressive anti-inflammatory treatments at disease onset, avoidance of infections, protease inhibition, and repurposing of drugs with known effects on protease balances. In the footsteps of the father of disease prevention and immunology, Edward Jenner, the definition of susceptible hosts for particular autoimmune diseases and the prevention of remnant epitope generation remain good advice. Therefore, research investments in prognostic biomarkers are crucial. The long-sought dream of universal cures for autoimmune diseases remains linked to central or peripheral tolerance induction. Perhaps research investments in new antimicrobials and nutritional control of microbiota with inhibitory effects on proteases and other enzymes ([Figure 2](#)) may serve as a double solution: the prevention of autoimmunization as well as the elimination of microbial epitope-generating and -modifying enzymes.

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Outstanding Questions

Do the differences between neutrophil and monocyte biology contribute in varying ways to the onset, progression, and resolution of the four phases of an autoimmune reaction? For instance, neutrophils quickly degranulate considerable amounts of uninhibited proteases, whereas monocytes/macrophages produce latent proteases and inhibitors, which are more dependent on the tissue context for proteolytic activity.

A vast amount of autoimmune research is invested in studying the roles of T cell subsets. Do subsets of B cells have differential functions and how do we define these for therapeutic purposes in specific autoimmune diseases? What are the (auto)antigens recognized by oligoclonal immunoglobulins in MS?

A step further is the differential function of myeloid cells, intensively studied in tumor immunology. How does the differentiation of the arbitrarily so-called M1 and M2 macrophages and N1 versus N2 neutrophil phenotypes contribute to specific autoimmune diseases?

What are the contributions of specific chemokines for the initiation, advance, maintenance, and obliteration of autoimmune processes and may this be exploited for therapy?

Similarly, what are the windows of opportunity to use the vast arrays of excellent protease inhibitors already in existence?

Besides proteins, other macromolecules, such as DNA and RNA, contribute to autoantibody formation. What are the roles of T cell subsets in this process? Can analogies to polysaccharide antigens provide insights?

The present REGA paradigm is still protein- and protease-centered. What are the roles played by oligosaccharide-based host antigens? As an example using the ABO blood group system, oligosaccharides represent excellent antigens, and immunoglobulin glycosylation is crucial in the regulation of autoimmune reactions. What will be the next breakthrough in glycobiology research for specific studies in autoimmunity?

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It is notoriously difficult to induce experimental autoimmune reactions in germ-free mice. With the present focus on microbiota research, what are the single microbes or combinations of those that can contribute to susceptibility for the development of EAE, collagen-induced arthritis, antibody-induced autoimmune reactions, etc.? In the event that in germ-free mice an autoimmune reaction is induced by a single bacterium, what alterations in innate and adaptive immune cells and molecules ensue?

The failure of T cell vaccination approaches have underlined the concept of epitope spreading. Besides MHC, what are the genetic and epigenetic determining factors of this phenomenon? If this question cannot be answered, is it possible to achieve a repertoire reset while preserving thymus and bone marrow function in a mammalian host?

Is it possible to turn the concept of T and B cell exhaustion into practice by eliminating pathogenic autoreactive clones, thereby avoiding autoantigen spreading?

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