In the spectrum of immunological diseases affecting various organs by inflammation and/or fibrosis, autoimmune reactions play an important role. Based on different studies both in humans as well as in animal models it becomes obvious that there is a broad range of pathologies that involve not only „primary“ autoimmune reactions but also other pathogenic mechanisms such as postinfectious and autoinflammatory processes. The heterogeneity within the immunological diseases may reflect the variable expression of autoinflammatory, autoimmune, and up to now unknown factors in disease development and manifestation. Based on histological and immunohistochemical examinations, IgG4-related sclerosing disease has been proposed as a novel clinicopathological entity with autoimmune phenomena but unknown etiology (chapter 1). The clarification of the etiopathological mechanisms is required to optimize prophylaxis, diagnostics and therapy. Especially, the application of novel and designer biological therapies (chapter 8) requires a better understanding of the processes that are involved in the genesis of immunological diseases. In chapter 2, some aspects of the role of epigenetic mechanisms and innate immunity in the pathogenesis of autoimmune diseases are described. Regardless of the underlying pathology, disease-associated autoantibodies are important biomarkers for the vast majority of non-organ and organ specific autoimmune diseases. However, to improve our understanding of these diseases and serological diagnostics it is necessary to search for novel autoantibodies, to further evaluate the real clinical relevance of known autoantibodies and to further develop and standardize the detection methods (chapters 3-5). Pathogenetic aspects as well as aspects of the serological diagnostics, including novel autoantibody specificities, novel methodologies and evaluation studies are presented for rheumatoid arthritis, systemic lupus erythematosus, antiphospholipid syndrome, systemic vasculitides, systemic sclerosis (chapter 6) and various organ specific diseases (chapter 7). In summary, the present volume highlights novel insights into the immune dysregulation, pathogenesis, serological diagnostics and biological therapies of autoimmune diseases.

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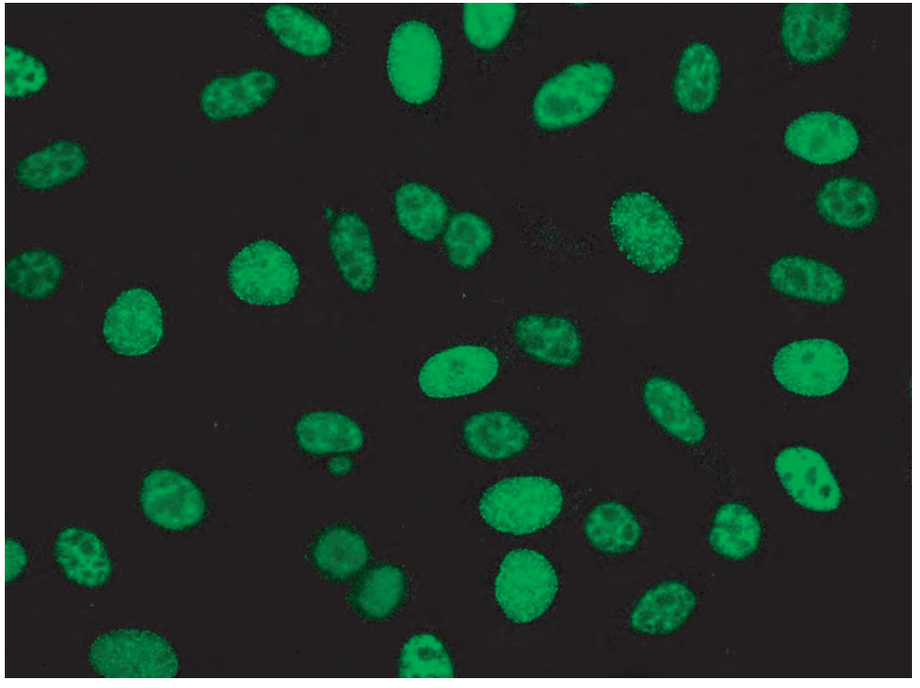

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Preface

In the spectrum of immunological diseases affecting various organs by inflammation and/or fibrosis, autoimmune reactions play an important role. Based on different studies both in humans as well as in animal models it becomes obvious that there is a broad range of pathologies that involve not only "primary" autoimmune reactions but also other pathogenic mechanisms such as postinfectious and autoinflammatory processes. The heterogeneity within the immunological diseases may reflect the variable expression of autoinflammatory, autoimmune, and up to now unknown factors in disease development and manifestation. For example, based on histological and immunohistochemical examinations, IgG4-related sclerosing disease has been proposed as a novel clinicopathological entity with autoimmune phenomena but unknown etiology. The clarification of the etiopathological mechanisms is required to optimize prophylaxis, diagnostics and therapy. Especially, the application of novel and designer biological therapies requires a better understanding of the processes that are involved in the genesis of immunological diseases. An important role in the pathogenesis of autoimmune diseases is discussed for epigenetic mechanisms and components of the innate immunity. The further exploration of those processes including the involved exogenous factors may offer novel prophylactic and therapeutic perspectives.

Regardless of the underlying pathology, disease-associated autoantibodies are important biomarkers for the vast majority of non-organ and organ specific autoimmune diseases. However, to improve our understanding of these diseases and serological diagnostics it is necessary to search for novel autoantibodies, to further evaluate the real clinical relevance of known autoantibodies and to further develop and standardize the detection methods. Pathogenetic aspects as well as aspects of the serological diagnostics, including novel autoantibody specificities, novel methodologies and evaluation studies for rheumatoid arthritis, systemic lupus erythematosus, antiphospholipid syndrome, systemic vasculitides, systemic sclerosis and various organ specific diseases are presented in this volume. We are sure that the novel insights into the immune dysregulation and pathogenesis described and discussed in this volume will stimulate novel concepts to improve diagnostics, prognostics and biological therapies of immune mediated diseases.

The editors

Proteinase 3 and its receptors: linking innate immunity to autoimmunity in ANCA-associated vasculitides

E. Csernok*

Department of Rheumatology, University Lübeck and Rheumaklinik Bad Bramstedt, Germany

Introduction

Proteinase 3 (PR3) is a multifunctional neutrophil-derived serine protease influencing cell cycle, differentiation, and cell death, and is the main target antigen of autoantibodies in ANCA-associated vasculitides (AAV), especially Wegener's granulomatosis (WG), known as antineutrophil cytoplasmic antibodies (PR3-ANCA). PR3-ANCA is thought to play a critical role in the pathogenesis of vascular damage in AAV. In contrast, it is not clear how the granulomatous inflammation, the hallmark of WG, is driven, and what is the relationship between granuloma and autoimmunity.

Current understanding of the molecular mechanisms by which PR3 regulates inflammatory processes and induces autoimmunity is still lacking. Recently, evidence shows that interactions of PR3 with two new molecules (protease-activated receptor-2: PAR-2 and Interleukin-32: IL-32) actively contribute to regulation of inflammation and immune functions in WG. This review mainly focuses on PR3-mediated dendritic cell (DC) activation and differentiation involving PAR-2 in WG.

*Corresponding author: Dr. Elena Csernok, PhD
Department of Rheumatology
University of Lübeck and Klinikum Bad Bramstedt
Oskar-Alexanderstr. 26
24576 Bad Bramstedt, Germany
E-mail: csernok@r-on-klinik.de

PR3 and dendritic cells

One of the key questions with respect to the pathophysiology of human autoimmune diseases is how autoreactivity to the particular autoantigen(s) is initiated. The selection of a self-molecule as a target for an autoantibody response might be the consequence of a direct pro-inflammatory interaction of the molecule with a receptor on a gateway immune cell, such as an immature DC (the gateway-receptor model) [1]. PR3 is an ideal candidate for this role as it is not expressed (or quickly inactivated by serine protease inhibitors) in the extracellular space of healthy tissue, however, its level increases during infection, trauma and tissue necrosis. A number of studies demonstrated that at sites of inflammation an increased amount of PR3 is detected in the extracellular space in WG [2, 3, 4]. Most importantly, this protein was most prominently present within the affected tissues of the upper respiratory tract (i.e., nasal granulomatous lesions), which is the place, were the first clinical symptoms of disease occur- and possibly, where autoimmunity is generated [5]. Indeed, in early granulomatous lesions of WG-patients we have found evidence of maturation of autoreactive B-cells, as suggested by ANCA-encoding VH genes [5]. Therefore, granulomatous lesions themselves could represent a (tertiary) lymphoid-like tissue in which the autoantigen is displayed under inflammatory conditions [18]. Furthermore, PR3 was detected on the cell surface of neutrophils and a high membrane PR3 expression is a risk factor for WG [6, 7]. As PR3 can be mobilised upon apoptosis independent from degranulation, expression of PR3 on the surface of apoptotic blebs and ectosomes may render PR3 as an antigenic target.

It was reported that PR3 activates oral epithelial cells through G-protein-coupled protease activating receptor 2 (PAR-2) and actively participates in the process of inflammation such as peridontitis [8]. Furthermore, PARs provide a system that detects tissue injury and triggers a set of cellular responses that contribute to various responses including inflammation [9, 10].

Therefore, we tested the hypothesis whether PR3 possess the capacity to interact and activate PAR-2-expressing antigen presenting cells (APC) and thereby potentially links this inflammatory activity to the initiation of an adaptive immune response.

We demonstrated that PR3 induces phenotypic and functional maturation of blood monocyte-derived iDCs. PR3-treated DCs express high levels of CD83, a DC-restricted marker of maturation, costimulatory molecules CD80 and CD86, and HLA-DR. Furthermore, they become fully competent antigen presenting cells and can induce stimulation of PR3-specific $CD4^+$ T cells, which produce INF-γ and drive the polarization towards a Th1 phenotype [11].

PR3 and PAR-2

We next examined the pathway of PR3-induced maturation of DCs, with special interest to the PR3-receptor(s). We demonstrated that interaction of PR3 with PAR-2 leads to DC activation and differentiation.

To study the cleavage profile of serine proteases PR3, HLE and CG we used a classical approach: a synthetic peptide corresponding to a region spanning the cleavage site of the PAR-2, residues 32–45 (^{32}SSKGRSLIGKVDGT^{45}), was HPLC-separated after the cleavage and analyzed by amino acid sequencing and MALDI mass spectrometry. The results show that PR3 can cleave the synthetic peptide after the valine residue at position 42 (V^{42}-D^{43}) which results in a C-terminal release of the activating peptide. Thus, PR3 has the potential to cleave the peptide on the opposite site of the thetered ligand (SLIGKV). In contrast, Uehara et al. reported that PR3 cleaves the PAR-2 peptide at the site R^{36}-S^{37} [8]. Differences in purity of the proteases may account for the divergent findings regarding the cleavage site of PR3.

Evidence suggests that the cleavage at the site V^{42}-D^{43} by PR3 may be functionally relevant: (1) a blocking antibody against PAR-2 inhibits the PR3-induced maturation of dendritic cells. (2) the principal mechanism of PAR-mediated activation is through Gαq-proteins, resulting in activation of phospholipase C (PLC). Therefore, the involvement of PAR-2 in DC maturation was further analysed by addition of a specific inhibitor of PLC in combination with PR3 or PAR-2 peptide agonist (PAR-2AP). It was demonstrated that the differentiation of DC by PR3 via PAR-2 activation uses the Gαq-proteins signaling pathway only partially; (3) PR3, but not HLE and CG, induced the expression of PAR-2 on DC, suggesting that this effect is PR3-specific; (4) the PAR-2 agonist peptide SLIGKV-NH2, corresponding to the PAR-2 tethered ligand, induced maturation of DC. PAR-2AP up-regulated the expression of CD83, HLA-DR, and costimulatory molecules on DC in similar intensity as compared to PR3, suggesting a similar mode of action; (5) HLE and CG digestion of the PAR-2 peptide resulted in different cleavages, but not at the activating site of PAR-2, suggesting that only the cleavage induced by PAR-2 is functionally relevant.

Our results suggested that DC maturation via PAR-2 activation by PR3 with Th1 polarisation may influence the immune response in the tissue microenvironment. In the setting of various non specific nasal tissue injuries (e.g., bacterial infection: *Staphylococcus*, drugs: cocaine), increased numbers of neutrophils that express "Wegener's autoantigen" at high levels are induced, providing the target to focus antigen-specific responses in tissue. PAR-2 may serve a physiological purpose similar to that of TLRs and senses endogenous "danger/alarm" signals in the environment, such as serine proteinase PR3, and its activation influences the development of both innate immune response, namely inflammation, and adaptive immune responses, and namely the decision of the immune system to respond to the self molecules. Thus, the primary role of PR3 as "danger signal" may alert

the immune system and may facilitate and promote tissue repair and restoration. Recently, a number of studies speculated that autoantigens may serve as "danger/alarm signals" and suggested a "beneficial role" of autoimmunity in tissue repair processes (Fig. 1).

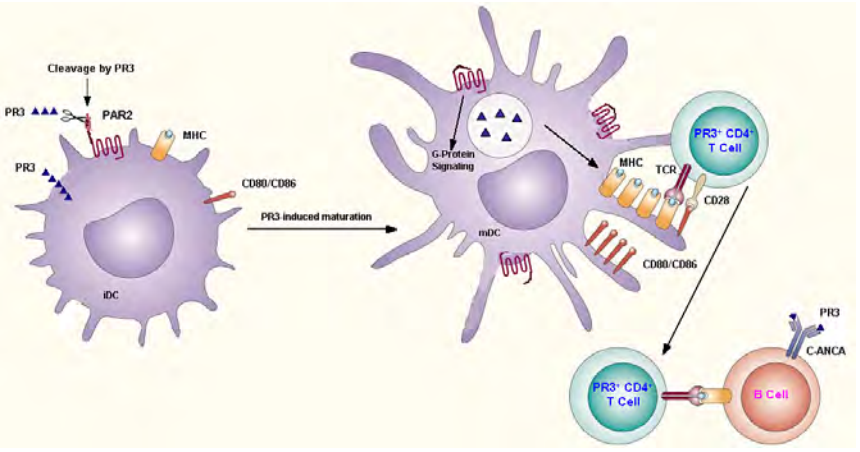

Figure 1. *The gate-way receptor model:* In WG, expression of PR3 in the extracellular space is increased. PR3 stimulates the expression of PAR-2 on DC and activates PAR-2 resulting in maturation of DC, as indicated by expression of CD80, CD83, CD86 and HLA-DR and these PR3-maturated DCs stimulate $CD4^+$ T cells to generate increased expression of IFN-γ. Hypothetically, T-cell activation by PR3-maturated DCs may finally promote the development of B-cells towards ANCA-producing plasma cells. Modified from [1].

PR3 and IL-32-alpha

Interestingly, PR3 exhibits a unique property regarding the interaction with interleukin-32, a recently discovered proinflammatory cytokine that induces TNF-α, IL-1β, IL-6 and 2 C-X-C chemokine family members involved in several autoimmune diseases [12]. PR3 is a specific IL-32α-binding protein, independent of its enzymatic activity. However, cleavage of IL-32 by enzymatically active PR3 enhances activities of this cytokine. Therefore, specific inhibition of PR3 activity to process IL-32 or neutralisation of IL-32 by inactive PR3 or its fragments may reduce the impact of IL-32 on inflammation and autoimmune disease [12]. However, at the moment it is unclear whether PR3 functions primarily as binding protein for endogenous IL-32α or cleaves IL-32α, resulting in biologically active fragments.

We are currently investigating IL-32 expression on nasal biopsy from WG pateints and circulating blood leukocytes and we detected a high IL-32-alpha intra-

cellular. Interestingly, IL32-alpha is partial colocalized with PR3 in the WG tissue (unpublished data).

Summary and conclusions

The described observations raise the attractive hypothesis that PR3 expression results in cleavage and activation of PAR-2 on membrane of immune cells with its proinflammatory effects, such as induction of IFN-γ production by $CD4^+$T cells. Since the IL-32 production is caspase1/IL-18/IFN-γ dependent [13], it is possible that the cleavage and activation of IL-32 by PR3 takes also place in DC which results in downstream inflammation. However, PR3 is also an IL-32 binding protein and the neutralising effect of soluble PR3, released from activated and/or dying neutrophils, on the IL-32 activity may represent a negative feedback mechanism at the inflammatory site. Thus, PR3 might have a dual effect in the pathogenesis of WG: first, it can act as an initiator of innate immunity at the frontline and second, PR3 might be involved in the negative feedback mechanisms that suppress ongoing inflammation. Presumably, in patients with genetic and immunoregulatory defects, tissue damage may initiate immune responses via PR3 that persist, despite repair of the damage, and culminate in inappropriate autoimmune, self destructive reactions, as seen in WG patients. Nasal carriage of *S. aureus,* that is associated with an increased rate of relapse [14], could trigger new activity in previously induced lesions.

References

[1] Plotz PH. The autoantibody repertoire: searching for order. Nat Rev Immunol. 2003; 3: 73–78.

[2] Braun MG, Csernok E, Gross WL, Muller-Hermelink HK. Proteinase 3, the target antigen of anticytoplasmic antibodies circulating in Wegener's granulomatosis. Immunolocalization in normal and pathologic tissues. Am J Pathol. 1991; 139: 831–838.

[3] Mrowka C, Csernok E, Gross WL, Feucht HE, Bechtel U, Thoenes GH. Distribution of the granulocyte serine proteinases proteinase 3 and elastase in human glomerulonephritis. Am J Kidney Dis. 1995; 25: 253–261.

[4] Bajema IM, Hagen EC, de Heer E, van der Woude FJ, Bruijn JA. Colocalization of ANCA-antigens and fibrinoid necrosis in ANCA-associated vasculitis. Kidney Int. 2001; 60: 2025–2030.

[5] Voswinkel J, Mueller A, Kraemer JA, Lamprecht P, Herlyn K, Holl-Ulrich K, Feller AC, Pitann S, Gause A, Gross WL. B lymphocyte maturation in Wegener's granulomatosis: a comparative analysis of VH genes from endonasal lesions. Ann Rheum Dis Nov 2005

[6] Csernok E, Ernst M, Schmitt W, Bainton DF, Gross WL. Activated neutrophils express proteinase 3 on their plasma membrane in vitro and in vivo. Clin Exp Immunol 1994; 95: 244–250.

[7] Witko-Sarsat V, Dalldorf FG, Hieblot C, Guichard J, Nusbaum P, Lopez S, et al. Presence of proteinase 3 in secretory vesicles: evidence of a novel, highly mobilizable intracellular pool distinct from azurophil granules. Blood 1999; 94: 2487–2496.
[8] Uehara A, Sugawara S, Muramoto K, Takada H. Activation of human oral epithelial cells by neutrophil proteinase 3 through protease-activated receptor-2. J Immunol. 2002; 169: 4594–4603.
[9] Fields RC, Schoenecker JG, Hart JP, Hoffman MR, Pizzo SV, Lawson JH. Protease-activated receptor-2 signaling triggers dendritic cell development. Am J Pathol. 2003; 162: 1817–1822
[10] Caugllin SR, Camerer E. Participation in inflammation. 2003. J Clin Invest; 111 (1): 25–27
[11] Csernok E, Ai M, Gross WL, Wicklein D, Petersen A, Lindner B, Lamprecht P, Holle JU, Hellmich B. Wegener's autoantigen induces maturation of dendritic cells and licenses them for Th1 priming via the protease-activated receptor-2 pathway. Blood 2006; 107: 4440–8.
[12] Novick D, Rubinstein M, Azam T et al., Proteinase 3 is an IL-32 binding protein. 2006. PNAS; 103 (9): 3316–3321
[13] Netea MG, Azam T, Lewis EC, et al., Mycobacterium tuberculosis induces IL32 production through a caspase-1/IL-18/INF-γ-dependent mechanism. 2006. Plos Med, 3 (8): 1310–1318
[14] Stegeman CA, Tervaert JW, Sluiter WJ et al., Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener's granulomatosis. 1994. Ann Intern Med; 120: 12–14

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The clinical paradox of esoteric and novel autoantibodies

M. J. Fritzler*

Faculty of Medicine, University of Calgary, Calgary, Canada

Abstract

Autoantibodies (Aab) are biomarkers found in most autoimmune diseases, but in contrast to genetic biomarkers that reputedly indicate disease predisposition, the role of Aab is much less clear: some are pathogenic, some are disease specific; others antedate the full clinical expression of disease and serve as predictors of disease outcome; some may provide protection against disease; and some serve as signatures of inciting agents of autoimmunity. Over the five decades that followed the first description of Aab in systemic autoimmune rheumatic diseases (SARD), a few Aab have become best known as diagnostic biomarkers but even fewer are used as classification criteria for SARD. Because of growing evidence that some Aab antedate the clinical diagnosis, significant effort is being expended on gathering evidence about their value as predictors of disease onset and outcome. The ever growing lists of Aab associated with SARD have presented significant challenges to physicians and laboratory clinicians alike. The rapid expansion of knowledge about Aab has led to assumptions that many, if not most, of the newer Aab have little clinical value and hence they are often relegated to a category of "esoteric" Aab. However, the clinical value of some of these Aab becomes clearer if the perspective is changed from that of viewing them in the context of a clinical cohort to the context of a serological cohort. Simply stated, the clinical value of many Aab is based on the notion that if they are relatively sensitive and/or specific markers for a given SARD, that they have diagnostic and prognostic value. However, the clin-

*Corresponding author: Prof. Marvin J. Fritzler, MD
Faculty of Medicine: HRB414
University of Calgary
3330 Hospital Dr NW
Calgary, Alberta, Canada T2N 4N1
E-mail: fritzler@ucalgary.ca

ical value of many esoteric Aab that are not sensitive markers of SARD becomes more apparent if disease associations are examined and certain diseases emerge as common constituents of serological cohorts. Hence, this contrast of disease associations between disease cohorts and serological cohorts can be regarded as a serological paradox when considering the clinical value of the broad spectrum of Aab that have been described to date.

Introduction

Systemic autoimmune rheumatic diseases (SARD) are characterized by the presence of circulating autoantibodies (Aab) directed to a variety of intra- and extracellular components. Historically, Aab have been used primarily to assist the clinician in detecting, diagnosing, classifying and following the clinical course of SARD. Not long after the discovery of the LE cell and antinuclear antibodies (ANA), studies were designed to determine if Aab were also involved in pathogenesis of their associated diseases. In part, these investigations were sparked by observations that Aab in organ specific autoimmune disease such as Grave's disease, Addison's disease, pernicious anemia and myasthenia gravis could be linked to the pathogenesis of these conditions [1]. A half century of extensive studies of the pathogenic role of Aab in SARD has been marked by progress but, in many cases, a direct pathogenic role of most Aab in SARD remains unknown or controversial.

As studies of Aab progressed, it became clear that they were not an exclusive feature of established disease because they were also seen in first degree relatives of patients, individuals with *forme fruste* disease, patients with apparent unrelated conditions such as infections and malignancy, and even in normal blood donors. This picture became more complex when it became known that Aab antedated the onset of clinical disease [1, 2].

Most SARD are characterized by a spectrum of Aab directed to a wide range of nuclear, cytoplasmic, cell membrane and extracellular components. The Aab targets include proteins, nucleic acids, nucleoproteins, phospholipids, glycoproteins, and glycolipids. In systemic lupus erythematosus (SLE) there are now over 150 [3, 4], in systemic sclerosis over 50 [5, 6] and in antiphospholipid syndrome over 30 [7] Aab described and the list continues to grow. The focus of this review will be the challenges encountered in understanding the clinical importance of Aab, particularly "esoteric" Aab that are uncommon or not considered to be specific for certain diseases (Table 1).

Autoantibody profiles: a challenge for diagnostic platforms

Very early in the study of SLE, it was obvious that an individual serum at any time during the clinical course of the disease contained multiple Aab that are typically directed to components of the same macromolecular complex [8, 9]. At

Table 1. Targets of esoteric autoantibodies*.

Localization	Autoantigens
Golgi complex	golgins-67, -97, 95/gm130, golgins-160, 245, giantin
Endosomes	early endosome antigen 1 (EEA1), cytoplasmic linker protein (CLIP170), lysobisphosphatidic acid (LBPA), GRASP-1
GW Bodies (processing bodies)	GW proteins (GW182, GW2, GW3), hAgo2, Ge-1/Hedls, RAP55/LSm15, LSm4
Centrosome	pericentrin, PCM-1, -2, ninein, mob-1
Proteasome	A3-HC9, Ki~p28g
Asssemblyosome — SMN complex	Sm, RNA helicase (Gu), fibrillarin, p80 coilin
Intracellular Exosome**	PM/Scl-75, -100, hCs14, hRrp4, 40, 41, 42
Extracellular Exosomes***	
Cell Membrane	Aquaporin 4****

* reviewed in [22, 24], ** see [97], *** see [70–74], **** see [70–74]

the same time, it was thought that sera from patients with polymyositis, systemic sclerosis (SSc), Sjögren's syndrome (SjS) and rheumatoid arthritis had a rather monospecific, if not narrow, autoantibody profile [10]. However, with the advent of multiplexed and multianalyte immunoassays, it has been clearly demonstrated that sera from these patients also commonly contain multiple Aab [11]. Support for the concept that multiple Aab are found in individual serum is supported by experimental and observational studies showing that Aab develop along a pathway described as intra- and inter-molecular epitope spreading [12–14].

The observation that SARD sera often contain multiple Aab has raised the question as to their clinical and/or pathological significance. In other words, does knowing that a patient with Raynaud's phenomenon has antibodies to RNA polymerase III and mitochondria add value to the clinical management of the patient? Or does knowing that a patient with a photosensitive skin rash, alopecia and arthritis has antibodies to Sm, chromatin and cyclic citrullinated peptides have any clinical relevance? The answers to these kinds of questions are, for the most part, unknown and this is due to several factors. First, the antinuclear antibody (ANA) test remains the screening assay of choice when physicians evaluate patients for a diagnosis of SARD. While the ANA screening test has many positive features, it is not the method of choice to identify multiple autoantibodies in an individual serum with high precision. Given the tremendous strides in identifying the molecular biology of many autoantigens described to date, the ability to identify multiple Aab in a single serum is now made possible by using multiplexed diagnostic plat-

forms [15, 16]. An important question is whether the indirect immunofluorescence (IIF) screening test has sufficient sensitivity to detect all clinically relevant Aab. To address this question we evaluated a cohort of sequential and unselected sera that were tested at a serum dilution of 1/160 as recommended by an expert committee [17] and that were negative in the HEp-2 IIF screening test and retested them by ALBIA (INOVA: ENA8) and found that 18 % had a positive result (unpublished data). Among the autoantibodies in this presumed Aab/ANA negative cohort, some were directed to ribosomal P protein, Jo-1 and Ro52. In recognition of this shortcoming of IIF screening tests, some laboratories have inverted the diagnostic algorithm by first screening sera with multiplexed technologies and then testing negative sera with an IIF assay. The cost-effectiveness of either approach requires thorough analysis.

To complicate the clinical diagnostic scenario further, it is widely known that many patients with one autoimmune condition often have, or develop, one or more additional autoimmune diseases (reviewed in [18]). A recent study that highlighted the importance of testing for multiple Aab found that approximately 50 % of patients with pernicious anemia had concurrent autoimmune thyroid disease [19]. In a recent study of SLE patients, clinically overt disease was found in only six percent but subclinical thyroid disease was identified in twelve percent and positive thyroid autoantibodies in the absence of thyroid disease in seventeen percent [20]. Further, thyroid Aab preceded the occurrence of clinical autoimmune thyroid disease in 70 % of these SLE patients. Thus, a major clinical challenge is the knowledge that in many cases the second autoimmune disease, whether it is Hashimoto's thyroiditis, celiac disease, or pernicious anemia, is undiagnosed. These observations and many others like them, point to the importance of detecting multiple Aab in a single serum sample.

In considering some of these challenges and the ideal diagnostic platform of the future, Bizzaro and his colleagues proposed that the ideal diagnostic platform would include the simultaneous detection of 25–30 Aab coupled with the detection of 2 or more immunoglobulin isotypes; a highly automated, high throughput system that had high analytical accuracy; and all being performed at a cost of 5–10 fold lower than that of conventional tests for these multiple Aab [21]. There is healthy skepticism in the industry that these parameters (particularly cost containment and kit pricing) can be met but in the face of rapidly escalating health care costs, economical and cost effective diagnostics will likely emerge the winners.

The autoantibody paradox

In the past, most prospective and retrospective Aab analyses have focused on the most common Aab such as dsDNA and anti-Sm in SLE; anti-topoisomerase I and anti-centromere in systemic sclerosis; anti-SSA/Ro and anti-SSB/La in Sjögren's syndrome; anti-Jo-1 and anti-PM/Scl in polymyositis; anti-CCP and rheumatoid

factor in RA; anti-cardiolipin and anti-β2 glycoprotein I in anti-phospholipid syndrome. Such studies are typically based on the perspective that only the most common and relatively specific Aab in disease cohorts are clinically relevant. Admittedly, certain Aab are rarely encountered in cohorts with established diagnoses and for that reason they have been referred to as 'esoteric' Aab (i.e. seen in <5 % of disease cohorts) (Table 1) [22, 23]. However, it may not be widely appreciated that in a diagnostic laboratory setting esoteric Aab are detected as commonly as many other more widely studied Aab [24]. To explore and elucidate the concept of the Aab paradox represented by the studies of esoteric Aab, we will highlight four different esoteric Aab, anti-Golgi, anti-CENP-F, anti-GW bodies and anti-aquaporin 4 (AQP4), and by examining the disease associations of serological cohorts rather than disease cohorts, shed light on the on the value of identifying these Aab in a clinical setting.

Anti-Golgi antibodies (AGA)

The Golgi complex is localized in the perinuclear region of most mammalian cells (Fig. 1) and is characterized by membranous stacks organized as distinct cis-, medial- and trans-Golgi networks [25–27]. The Golgi complex has a prominent

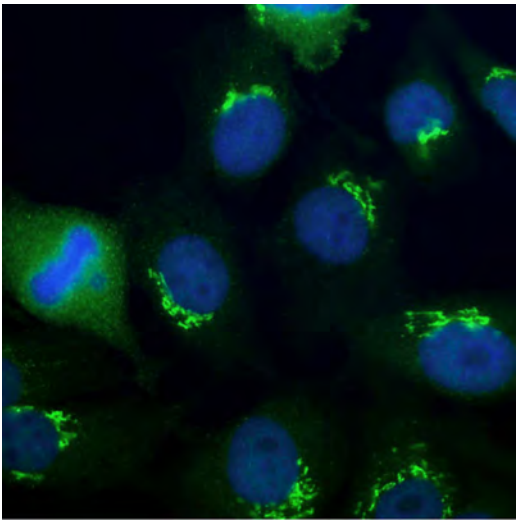

Figure 1. IIF of human autoantibodies to golgins in the Golgi complex are characterized as intense IIF lamellar and nearby speckled staining in region on one side and adjacent to the nucleus of HEp-2 cells. Original magnification × 600.

function in the processing, transporting, and sorting of newly synthesized proteins from the rough endoplasmic reticulum.

In the last two decades the identity of Golgi complex autoantigens has been elucidated and lead to the identification of a unique family of proteins, referred to as golgins [28]. The golgin autoantigens include giantin/macrogolgin/GCP372, golgin-245/p230, golgin-160/GCP170, golgin-95/gm130, golgin-97, 115, and golgin-67 [25–27, 29–33]. All of the golgins, except giantin/macrogolgin and perhaps golgin-67, are peripheral Golgi components bound on the cytoplasmic face of Golgi membranes. Giantin/macrogolgin has a single trans-membrane domain in the C-terminus but the majority of the molecule projects into the cytosol [34]. Golgin-245 and golgin-97 were localized to the *trans*-Golgi compartment [35] and gm130/golgin-95 was reported in the *cis*-Golgi compartment [36]. Golgin-245 and golgin-97/GM130 attach to Golgi membranes through a GRIP domain in their C-termini [37].

Unlike many human autoantigens that are found in cell surface blebs during apoptosis [38], the Golgi complex and other cytoplasmic organelles (i.e. mitochondria, lysosomes, endosomes, peroxisomes) co-clustered to a crescentic region of a misshaped "half-moon" nucleus [39]. In addition, a viral etiology in the generation of AGA was implied in studies showing that mice infected with a certain strain of the lactate dehydrogenase-elevating virus produce AGA [40].

Anti-Golgi complex autoantibodies (AGA) were initially identified in the serum of a SjS patient with lymphoma [41] and this was followed by other reports that described AGAs in SjS [42], SLE [43], rheumatoid arthritis [44], mixed connective tissue disease [45], Wegener's granulomatosis [46] and HIV infection [29, 47]. Immunoblotting and immunoprecipitation studies have shown that the proteins recognized by human AGA are remarkably heterogeneous [48] and suggests that other Golgi antigen targets are yet to be identified. In a study of 80 sera, the frequency of AGA was correlated with the molecular masses of the golgins [49]. For example, Aab to giantin/macrogolgin, the highest molecular weight golgin, were the most frequent, being found in 50 % of the AGA sera. By contrast, antibodies to golgin 97 were the least common, being found in only approximately 4 % of the AGA sera. The most reactive of the giantin/macrogolgin epitopes were those that encompass the C-terminal trans-membrane domain [49].

Although AGA are generally considered to be rare, at the Advanced Diagnostics Laboratory at the University of Calgary, they were found to be at least as common as antibodies to Sm [22]. The importance of AGA in clinical practices highlights the paradox discussed above. First, AGA are quite rare (<1 %) in unselected SARD sera when serological cohorts are studied, but up to 30 % of AGA positive sera are from SjS and patients with other SARD (reviewed in [24, 50]). Evidence indicating that AGA associate with a subset of SjS or other diseases has yet to be proven. However, it is of interest that high titer AGA have been suggested to constitute an

early sign of systemic autoimmune diseases even in the absence of clear clinical manifestations [51].

Anti-CENP-F antibodies

Historically, we first became interested in Aab to centromere protein (CENP)-F when we published our studies of centromere related patterns of IIF produced by a subset of SSc patients with the CREST or limited cutaneous variant of the disease [52–54]. During the course of those studies we became aware that several IIF patterns resembled the typical CENP pattern but had remarkable differences [55]. One of these patterns we tentatively named NSP-II, which at first glance resembled anti-CENPs, but the staining was different from antibodies to CENP-A or CENP-B, which typically stain both interphase nuclei and mitotic chromatin. The NSP-II pattern did not have staining of interphase cells but gave a fairly typical CENP pattern in metaphase cells (Fig. 2). In addition, there was often staining of cells in anaphase, telophase (stem body) and cells in prometaphase (G2-G3) [56]. Eventually, the target autoantigen was identified as the CENP-F protein [57, 58]. CENP-F (also called mitosin) is a large (~400 kDa) coiled-coil, nuclear matrix

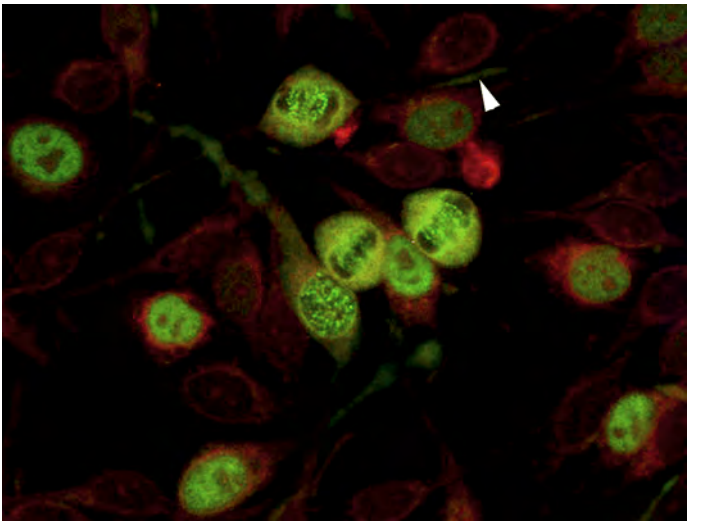

Figure 2. Typical indirect immunofluorescence staining pattern of CENP-F autoantibodies characterized by speckled staining of metaphase cells. Unlike antibodies to CENP-A/CENP-B, there is no staining of the majority of interphase cells. Staining of the midbody (white arrow) of telophase cells is seen in some sera but is not a universal feature of all CENP-F positive sera. Cells are counterstained red with Evan's blue. Photograph courtesy of Wendy Pollock, University of Melbourne & Gribbles Pathology, Australia.

protein that plays a role in the kinetochore-mediated mitotic functions, participates in the regulation of cell division, and is used as a proliferation marker of malignant cell growth in clinical and research laboratories (reviewed in [59]).

CENP-F Aab can be detected by special studies that utilize immunodominant peptides [60], which we have adapted to the ALBIA platform. Initial clinical studies indicated that approximately 50 % of patients that harbor this Aab have a malignancy [57, 60] but more recent studies in our laboratory indicate that the prevalence of malignancy in this serological cohort is much higher, around 80 % (unpublished data). In a 2005 publication, Bencimon and colleagues reported the prevalence and specificity of anti-CENP-F Aab in 347 non-Hodgkin's lymphoma (NHL) patients before they received any therapeutic intervention. Using a radioimmune assay (RIA) they found that 7.2 % of NHL patients and 1.3 % control patients had anti-CENP-F Aab as determined by RIA. By IIF, 2.9 % of NHL patients displayed the CENP-F or CENP-F-like pattern, whereas none in the control group did. These data demonstrate that a significant incidence of anti-CENP-F Aab was observed in NHL before any treatment and that RIA has much higher sensitivity but lower specificity than IIF. We have similar experience in our laboratory: an analysis of a cohort of various malignancies (lymphoma, breast and prostate cancer, melanoma) in an ALBIA that used the two immunodominant fragments of CENP-F [60] found that the prevalence of anti-CENP-F was 20 % but there was no association with any one malignancy or stage of the disease. In addition, only ~50 % of sera with reactivity as detected by ALBIA had detectable CENP-F IIF staining (unpublished results). The key issue in utilizing and understanding CENP-F is that when cohorts of individual malignancies are surveyed for CENP-F Aab, the frequency is generally low (< 20 %) but when a cohort of anti-CENP-F patients is surveyed, at least 50 % have a malignancy.

Anti-GWB antibodies

GW bodies (GWBs) are unique cytoplasmic structures involved in messenger RNA (mRNA) processing and RNA interference (RNAi). GWBs contain mRNA, components of the RNA-induced silencing complex (RISC), microRNA (miRNA), Argonaute proteins, the Ge-1/Hedls protein and other enzymes involving mRNA degradation [61, 62], many of which are autoantibody targets [63–66]. Sera with anti-GWB produce a typical cytoplasmic discrete speckled IIF pattern on HEp-2 and most other mammalian tissue culture cells (Fig. 3). A study to identify the GWB autoantigens targeted by 55 anti-GWB sera by ALBIA and immunoprecipitation of recombinant proteins indicated that Aab in this cohort of anti-GWB sera were directed against Ge-1/Hedls (58 %), GW182 (40 %) and Ago2 (16 %) [66]. Clinical data indicated that the most common clinical presentations were neurological symptoms (i.e. ataxia, motor and sensory neuropathy) (33 %), SjS (31 %) and the remainder had a variety of other diagnoses that included SLE, RA and

primary biliary cirrhosis (PBC). Although these studies of an anti-GWB serology cohort indicated that Sjögren's syndrome was one of the common diagnostic categories, a study of a cohort of a clinically-defined SjS cohort failed to identify a single patient with anti-GWB (unpublished data). Similarly, IIF studies of SLE and PBC cohorts indicated that less than 10 % of sera have anti-GWB antibodies (unpublished data and [67]).

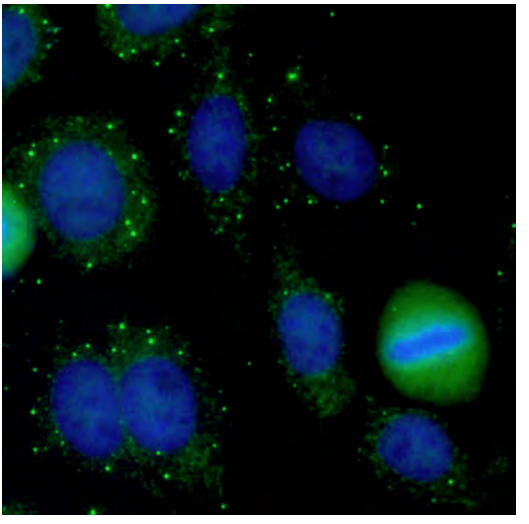

Figure 3. Autoantibodies to GW bodies are characterized as numerous cytoplasmic discrete speckles that are distributed throughout the cytoplasm of HEp-2 cell substrates (Immuno-Concepts). GWBs mark the cellular sites for mRNA processing via the microRNA and other pathways. Original magnification × 400.

Anti-Aquaporin 4 antibodies

The discovery of a specific autoantibody response directed against aquaporin-4 (AQP-4) in Devic's disease, a disease also known as opticospinal multiple sclerosis and, most commonly, neuromyelitis optica (NMO), [68–70] has been a significant step forward in defining, understanding the pathogenesis and giving a rational basis for therapeutic intervention of this condition (reviewed in [70–74]). NMO is a neurologic disease characterized by severe optic neuritis and transverse myelitis and attended by high morbidity and mortality. Of note, as implied in the name as 'opticospinal multiple sclerosis', NMO has features that overlap with multiple sclerosis (MS). Thus, an early and accurate diagnosis of NMO is extremely important because the optimum treatment for MS and NMO can differ considerably.

Aquaporin-4 (AQP4) is a water channel protein that is predominantly expressed in brain and spinal cord and evidence from clinical and pathological observations strongly supports the notion that AQP4 autoantibodies play a major role in the pathogenesis of NMO. For example, the pathological hallmark of NMO is a selective and characteristic deposition of immunoglobulins and complement on astrocytes at the *glia limitans,* which is accompanied by destruction and loss of glial fibrillary acidic protein and AQP-4 positive astrocytes followed by demyelination and eventually global tissue destruction [71, 75]. Of note, the distribution of NMO lesions in the brain and spinal cord correlates with the tissue distribution of AQP-4 expression. A recent immunogenetic study of Japanese patients showed that the frequency of the HLA-DPB1*0501 allele was significantly increased in anti-AQP4 antibody-positive patients (89.5 %, odds ratio = 4.8; 95 % confidence interval = 1.6–14.3, $n = 38$, $P = 0.032$) compared with controls (64.0 %, $n = 125$ T) [76]. Other evidence supporting an Aab-mediated disease is that clinical therapies designed to reduce the Aab load through plasmapheresis [75, 77], and/or targeting B lymphocytes [78], seem to be effective in alleviating some signs and symptoms of NMO. Taken together, this evidence supports the concept that NMO is an Aab-mediated autoimmune disease, although direct proof of the pathogenic role of AQP-4 antibodies or their temporal relationship to the disease has yet to be demonstrated.

In 2004, Lennon et al described an NMO IgG antibody using IIF on mouse cerebellum sections that showed a characteristic pattern of staining around microvessels, the pia, and Virchow-Robin spaces [68]. This assay was 58 % to 73 % sensitive and 91 % to 100 % specific for NMO. Since then, and following the discovery that AQP4 was the target antigen, a number of immunoassays have been developed to detect AQP4 antibodies: radioimimmunoprecipitation assay (RIPA) [70], fluoroimmunopreciptiation (FIPA) [79, 80], Elisa [81] and immunofluorescence utilizing cell based substrates transfected with the AQP4 cDNA [82, 83]. A study that compared the performance of some of these assays concluded that a cell based assay had higher sensitivity than the other assays [80]. Since the native form of the protein in western blots or recombinant full length or truncated proteins are poorly reactive, it is thought that the reactive AQP4 epitope is conformational or requires tertiary structure expression such as orthogonal arrays [84, 85]. Accordingly, the reactive portion of the protein has been localized to the third extracellular loop [85]. In an unpublished study, we used SPOT technology and previously published approaches [66, 86] that synthesize overlapping 15 mer peptides representing the full length AQP4 but no significant reactivity to these short peptides could be identified in four human NMO sera. We have also used a cell based assay wherein tissue culture cells are transfected with AQP4 (a gift of Euroimmun, Luebeck, Germany) (Fig. 4) and found high sensitivity (80 %) and specificity (90 %) in a small cohort of NMO and MS sera, results that are consistent with observations on cell based assays in other laboratories [83]. The Aab

titers on these substrates were > 1/640 and we also found that this cell based assay had higher sensitivity than other immunofluorescence based assays using human optic nerve or cerebellum (unpublished). A particular challenge in using organ or tissue sections and cell based assays is to discriminate AQP4 antibodies from other autoantibodies that can coexist in the same sera, particularly in SLE and SjS patients. However, we found that by using a rabbit anti-AQP4 antibody in a co-localization study, that the AQP4 reactivity can be distinguished from other Aab (Fig. 4).

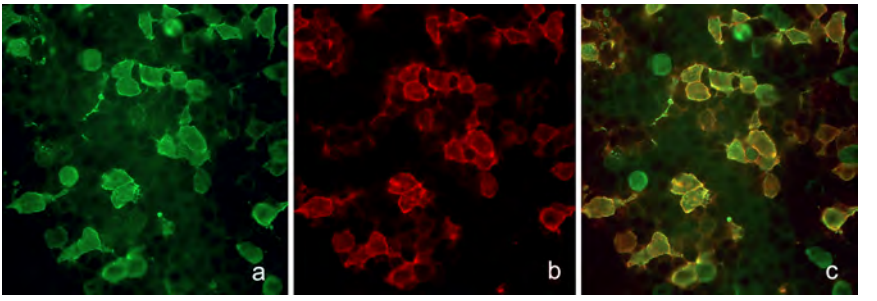

Figure 4. Autoantibodies to aquaporin 4 (AQP4) can be detected by indirect immunofluorescence on a cell line transfected with the corresponding cDNA (Euroimmun). A serum from a SLE patient with neuromyelitis optica reacts (panel a) with the AQP4 transfected cells that are specifically marked by rabbit antibodies to AQP4 (panel b). The two patterns of staining overlap as shown in the merged panel c. Cells stained by the SLE serum but not the rabbit anti-AQP4 represent cells reacting with other autoantibodies in the patient's serum. Original magnification × 400.

While most attention has focused on anti-AQP4 in NMO, these antibodies have been anecdotally described in other conditions such as SjS [87, 88], SLE [89] and SLE associated with anti-phospholipid antibodies [90], myasthenia gravis [91, 92], gluten enteropathy [93] and following herpes zoster infection [94]. However, as with other esoteric Aab, it is important to note that in disease cohorts of conditions like SLE, SjS and MS this Aab is rare but when serological anti-AQP4 cohorts are examined, the antibody is remarkably specific and sensitive for longitudinal (multi-segmental) transverse myelitis and/or optic neuritis.

In summary, antibodies to AQP4 represent one of the more important recent breakthroughs in identifying a target autoantigen in NMO and allow a more accurate diagnosis of transverse myelitis seen in the setting of SLE [89, 95], SjS [88, 96]. The importance of this discovery is that this Aab is likely pathogenic and although the frequency of anti-AQP4 is remarkably low in SLE, SjS, MS and other diseases, a serological cohort of anti-AQP4 patients have a very high (> 80 %) frequency of NMO, multisegmental neuromyelitis optica or related neurological problems.

Conclusion

In conclusion, studies focused on Aab that are commonly seen in disease cohorts could overlook potentially important biomarkers for SARD and other autoimmune diseases. Equally important, a clear understanding of the clinical associations of esoteric Aab is of critical importance because the diagnostic laboratory must be able to comment on their clinical relevance. Prospective and retrospective studies are urgently needed to determine the association of diseases with these serological cohorts. Such studies must be attended by the continued development of Aab assays in multiplexed platforms that facilitate their detection in SARD and other autoimmune sera.

References

[1] Conrad K, Schlösser W, Fritzler MJ. The predictive relevance of autoantibodies. In: From Etiopathogenesis to the Prediction of Autoimmune Diseases: Relevance of Autoantibodies.Langerich, Germany: Pabst Scientific Publishers; 2007. p. 16–31.
[2] Hoffman IE, Peene I, Meheus L, Huizinga TW, Cebecauer L, Isenberg D, et al. Specific antinuclear antibodies are associated with clinical features in systemic lupus erythematosus. Ann Rheum Dis 2004; 63: 1155–8.
[3] Sherer Y, Gorstein A, Fritzler MJ, Shoenfeld Y. Autoantibody explosion in systemic lupus erythematosus. Semin Arthritis Rheum 2004; 34: 501–37.
[4] Sherer Y, Shoenfeld Y. Autoantibody explosion in lupus — 155 different autoantibodies in SLE. Lupus 2007; 16 (suppl): 42.
[5] Steen VD. Autoantibodies in systemic sclerosis. Semin Arthritis Rheum 2005; 35: 35–42.
[6] Walker JG, Fritzler MJ. Update on autoantibodies in systemic sclerosis. Curr Opin Rheumatol 2007; 19: 580–91.
[7] Shoenfeld Y, Twig G, Katz U, Sherer Y. Autoantibody explosion in antiphospholipid syndrome. J Autoimmun 2008; 30: 74–83.
[8] Craft J, Hardin JA. Linked sets of antinuclear antibodies: what do they mean? J Rheumatol 1987; 14(suppl.): 106–9.
[9] Theofilopoulos AN. The basis of autoimmunity: Part I Mechanisms of abberant self-recognition. Immunol Today 1995; 16: 90–8.
[10] Nakamura RM, Tan EM. Autoantibodies to nonhistone nuclear antigens and their clinical significance. Hum Pathol 1983; 14: 392–400.
[11] Troyanov Y, Targoff IN, Tremblay JL, Goulet JR, Raymond Y, Senécal JL. Novel classification of idiopathic inflammatory myopathies based on overlap syndrome features and autoantibodies: analysis of 100 French Canadian patients. Medicine (Baltimore) 2005; 84: 231–49.
[12] Arbuckle MR, Gross T, Scofield RH, Hinshaw LB, Chang AC, Taylor FB, et al. Lupus humoral autoimmunity induced in a primate model by short peptide immunization. J Invest Med 1998; 46: 58–65.
[13] Harley JB, James JA. Autoepitopes in lupus. J Lab Clin Med 1995; 126: 509–16.

[14] Deshmukh US, Bagavant H, Lewis J, Gaskin F, Fu SM. Epitope spreading within lupus-associated ribonucleoprotein antigens. Clin Immunol 2005; 117: 112–20.
[15] Fritzler MJ. Advances and applications of multiplexed diagnostic technologies in autoimmune diseases. Lupus 2006; 15: 422–7.
[16] Fritzler MJ, Fritzler ML. Microbead-based technologies in diagnostic autoantibody detection. Expert Opin Med Diag 2009; 3: 81–9.
[17] Tan EM, Feltkamp TEW, Smolen JS, Butcher B, Dawkins R, Fritzler MJ, et al. Range of antinuclear antibodies in "healthy" individuals. Arthritis Rheum 1997; 40: 1601–11.
[18] Toh B-H, Whittingham S, Alderuccio F. Gastritis and Pernicious Anemia. In: The Autoimmune Diseases. Fourth ed. New York: Elsevier Academic Press; 2006. p. 527–46.
[19] Lahner E, Norman GL, Severi C, Encabo S, Shums Z, Vannella L, et al. Reassessment of Intrinsic Factor and Parietal Cell Autoantibodies in Atrophic Gastritis With Respect to Cobalamin Deficiency. Am J Gastroenterol 2009 in press.
[20] Appenzeller S, Pallone AT, Natalin RA, Costallat LT. Prevalence of thyroid dysfunction in systemic lupus erythematosus. J Clin Rheumatol 2009; 15: 117–9.
[21] Bizzaro N, Tozzoli R, Shoenfeld Y. Are we at a stage to predict autoimmune rheumatic diseases? Arthritis Rheum 2007; 56: 1736–44.
[22] Stinton LM, Eystathioy T, Selak S, Chan EKL, Fritzler MJ. Autoantibodies to protein transport and messenger RNA processing pathways: Endosomes, lysosomes, Golgi complex, proteasomes, assemblyosomes, exosomes and GW Bodies. Clin Immunol 2004; 110: 30–44.
[23] Stinton LM, Fritzler MJ. A clinical approach to autoantibody testing in systemic autoimmune rheumatic disorders. Autoimmun Rev 2007; 7: 77–84.
[24] Fritzler MJ, Stinton LM, Chan EKL. Autoantibodies to cytoplasmic autoantigens in endosomes, exosomes and the Golgi complex. In: From Etiopathogenesis to the Prediction of Autoimmune Diseases: Relevance of Autoantibodies. 5 ed. Lengerich, Germany: Pabst Science Publishers; 2007. p. 194–209.
[25] Chan EKL, Fritzler MJ. Golgins: coiled-coil-rich proteins associated with the Golgi complex. Available at: http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0717-34581998000200001&lng=en&nrm=iso.
[26] Chan EKL, Fritzler MJ. Autoantibodies to Golgi apparatus antigens. In: Pathogenic and Diagnostic Relevance of Autoantibodies. Proceedings 4th Dresden Symposium on Autoantibodies. Scottsdale, AZ: Pabst Scientific Publishers; 1998. p. 85–100.
[27] Renier G, Fritzler MJ, Chevailler A. Golgi apparatus autoantibodies. In: Autoantibodies.The Netherlands: Elsevier Science B.V.; 1996. p. 325–30.
[28] Barr FA, Short B. Golgins in the structure and dynamics of the Golgi apparatus. Curr Opin Cell Biol 2003; 15: 405–13.
[29] Seelig HP, Schranz P, Schroter H, Wiemann C, Renz M. Macrogolgin-A new 376 kD Golgi complex outer membrane protein as target of antibodies in patients with rheumatic diseases and HIV infections. J Autoimmun 1994; 7: 67–91.
[30] Fritzler MJ, Hamel JC, Ochs RL, Chan EKL. Molecular characterization of two human autoantigens: Unique cDNAs encoding 95- and 160-kD proteins of a putative family in the Golgi complex. J Exp Med 1993; 178: 49–62.
[31] Fritzler MJ, Lung C-C, Hamel JC, Griffith K, Chan EKL. Molecular characterization of golgin-245: A novel Golgi complex protein containing a granin signature. J Biol Chem 1995; 270: 31262–8.

[32] Griffith KJ, Chan EKL, Hamel JC, Miyachi K, Fritzler MJ. Molecular characterization of a novel 97 kDa Golgi complex autoantigen recognized by autoimmune antibodies from patients with Sjögren's syndrome. Arthritis Rheum 1997; 40: 1693–702.
[33] Eystathioy T, Jakymiw A, Fujita DJ, Fritzler MJ, Chan EKL. Human autoantibodies to a novel Golgi protein golgin-67: high similarity with golgin-95/gm 130 autoantigen. J Autoimmun 1999; 14: 179–87.
[34] Seelig HP, Schranz P, Schroter H, Wiemann C, Griffiths G, Renz M. Molecular genetic analyses of a 376-kilodalton Golgi complex membrane protein (Giantin). Mol Cell Biol 1994; 14: 2564–76.
[35] Erlich R, Gleeson PA, Campbell P, Dietzsch E, Toh B-H. Molecular characterization of *trans*-Golgi p230. J Biol Chem 1996; 271: 14: 8328–37.
[36] Nakamura N, Rabouille C, Watson R, Nilsson T, Hui N, Slusarewicz P, et al. Characterization of a cis-Golgi matrix protein, GM130. J Cell Biol 1995; 131: 1715–26.
[37] Munro S, Nichols BJ. The GRIP domain — a novel Golgi-targeting domain found in several coiled-coil proteins. Current Biology 1999; 9: 377–80.
[38] Mahoney JA, Rosen A. Apoptosis and autoimmunity. Curr Opin Immunol 2005; 17: 583–8.
[39] Nozawa K, Fritzler MJ, Takasaki Y, Wood MR, Chan EK. Co-clustering of Golgi complex and other cytoplasmic organelles to crescentic region of half-moon nuclei during apoptosis. Cell Biol Int 2009; 33: 148–57.
[40] Nozawa K, Fritzler MJ, Ikeda K, Takasaki Y, Satoh M, Chan EKL. Differential anti-Golgi complex autoantibody production following murine lastate dehydrogenase-elevating virus infection. Immunopharmacology and Immunotoxicology 2008; 30: 13–25.
[41] Rodriquez JL, Gelpi C, Thomson TM, Real FJ, Fernandez J. Anti-Golgi complex antibodies in a patient with Sjögren's syndrome and lymphoma. Clin Exp Immunol 1982; 49: 579–603.
[42] Blaschek MA, Pennec YL, simitzis AM, Le Goff P, Lamour A, Kerdraon G, et al. Anti-Golgi complex autoantibodies in patients with primary Sjögren's syndrome. Scand J Rheumatol 1988; 17: 291–6.
[43] Fritzler MJ, Etherington J, Sokoluk C, Kinsella TD, Valencia DW. Antibodies from patients with autoimmune disease react with a cytoplasmic antigen in the Golgi apparatus. J Immunol 1984; 132: 2904–8.
[44] Hong HS, Morshed SA, Tanaka S, Fujiwara T, Ikehara Y, Nishioka M. Anti-Golgi antibody in rheumatoid arthritis patients recognizes a novel antigen of 79 kDa (Doublet) by Western Blot. Scand J Immunol 1993; 36: 785–92.
[45] Rossie KM, Piesco NP, Charley MR, Oddis CV, Steen VD, Fratto J, et al. A monoclonal antibody recognizing Golgi apparatus produced using affinity purified material from a patient with connective tissue disease. Scand J Rheumatol 1992; 21: 109–15.
[46] Mayet WJ, Hermann E, Csernok E, Knuth A. A human renal cancer line as a new antigen source for the detection of antibodies to cytoplasmic and nuclear antigens in sera of patients with Wegener's granulomatosis. J Immunol Meth 1991; 143: 57–68.
[47] Gentric A, Blaschek M, Julien C, Jouquan J, Pennec Y, Berthelot JM, et al. Nonorgan-specific autoantibodies in individuals infected with Type 1 human immunodeficiency virus. Clin Immunol Immunopathol 1991; 59: 487–94.
[48] Kooy J, Toh BH, Gleeson PA. Heterogeneity of human anti-Golgi auto-antibodies: Reactivity with components from 35 to 260 kDa. Immunol Cell Biol 1994; 72: 123–7.

[49] Nozawa K, Fritzler MJ, Von Mühlen CA, Chan EKL. Giantin is the major Golgi autoantigen in human anti-Golgi complex sera. Arthritis Res Ther 2004; 6: R95-R102.
[50] Nozawa K, Fritzler MJ, Chan EK. Unique and shared features of Golgi complex autoantigens. Autoimmun Rev 2005; 4: 35–41.
[51] Bizzaro N, Pasini P, Ghirardello A, Finco, B. High anti-Golgi autoantibody levels: An early sign of autoimmune disease? Clin Rheumatol 1999; 18: 346–8.
[52] Fritzler MJ, Kinsella TD. The CREST syndrome: A distinct serologic entity with anticentromere antibodies. Am J Med 1980; 69: 520–5.
[53] Tan EM, Rodnan GP, Garcia I, Moroi Y, Fritzler MJ, Peebles C. Diversity of antinuclear antibodies in progressive systemic sclerosis: Anti-centromere antibody and its relationship to CREST syndrome. Arthritis Rheum 1980; 23: 617–25.
[54] Moroi Y, Peebles C, Fritzler MJ, Steigerwald J, Tan EM. Autoantibody to centromere (Kinetochore) in scleroderma sera. Proc Natl Acad Sci USA 1980; 77: 1627–31.
[55] Fritzler MJ, Valencia DW, McCarty GA. Speckled pattern antinuclear antibodies resembling anticentromere antibodies. Arthritis Rheum 1984; 27: 92–6.
[56] Liao H, Winkfein RJ, Mack G, Rattner JB, Yen TJ. CENP-F is a protein of the nuclear matrix that assembles onto kinetochores at late G2 and is rapidly degraded after mitosis. J Cell Biol 1995; 130: 507–18.
[57] Casiano CA, Humbel RL, Peebles C, Covini G, Tan EM. Autoimmunity to the cell cycle-dependent centromere protein $p330^{d/CENP\text{-}F}$ in disorders associated with cell proliferation. J Autoimmunity 1995; 8: 575–86.
[58] Rattner JB, Rao A, Fritzler MJ, Valencia DW, Yen TJ. CENP-F is a .ca 400 kDa kinetochore protein that exhibits a cell-cycle dependent localization. Cell Motil Cytoskelton 1993; 26: 214–26.
[59] Varis A, Salmela AL, Kallio MJ. CENP-F (mitosin) is more than a mitotic marker. Chromosoma 2006; 115: 288–95.
[60] Rattner JB, Rees J, Whitehead CM, Casiano CA, Tan EM, Humbel R-L, et al. High frequency of neoplasia in patients with autoantibodies to centromere protein CENP-F. Clin Invest Med 1997; 20: 308–19.
[61] Jakymiw A, Pauley KM, Li S, Ikeda K, Lian S, Eystathioy T, et al. The role of GW/P bodies in RNA processing and silencing. J Cell Sci 2007; 120: 1317–23.
[62] Eulalio A, Behm-Ansmant I, Izaurralde E. P bodies: at the crossroads of post-transcriptional pathways. Nat Rev Mol Cell Biol 2007; 8: 9–22.
[63] Eystathioy T, Chan EKL, Yang Z, Takeuchi K, Mahler M, Luft LM, et al. Clinical and serological associations of autoantibodies to a novel cytoplasmic autoantigen, GW182 and GW bodies. J Mol Med 2003; 81: 811–8.
[64] Jakymiw A, Ikeda K, Fritzler MJ, Reeves WH, Satoh M, Chan EK. Autoimmune targeting of key components of RNA interference. Arthritis Res Ther 2006; 8: R87.
[65] Yang WH, Bloch DB. Probing the mRNA processing body using protein macroarrays and 'autoantigenomics'. RNA 2007; 13: 704–12.
[66] Bhanji R, Eystathioy T, Chan EKL, Bloch DB, Fritzler MJ. Clinical and Serological Features of Patients with Autoantibodies to GW/P Bodies. Clin Immunol 2007; 123: 247–56.
[67] Bloch DB, Yu JH, Yang WH, Graeme-Cook F, Lindor KD, Viswanathan A, et al. The cytoplasmic dot staining pattern is detected in a subgroup of patients with primary biliary cirrhosis. J Rheumatol 2005; 32: 477–83.

[68] Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet 2004; 364: 2106–12.
[69] Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 2005; 202: 473–7.
[70] Paul F, Jarius S, Aktas O, Bluthner M, Bauer O, Appelhans H, et al. Antibody to aquaporin 4 in the diagnosis of neuromyelitis optica. PLoS Med 2007; 4: e133.
[71] Bradl M, Lassmann DH. Anti-Aquaporin-4 Antibodies in Neuromyelitis Optica: How to Prove their Pathogenetic Relevance? Int MS J 2008; 15: 75–8.
[72] Graber DJ, Levy M, Kerr D, Wade WF. Neuromyelitis optica pathogenesis and aquaporin 4. J Neuroinflammation 2008; 5: 22.
[73] Hinson SR, Roemer SF, Lucchinetti CF, Fryer JP, Kryzer TJ, Chamberlain JL, et al. Aquaporin-4-binding autoantibodies in patients with neuromyelitis optica impair glutamate transport by down-regulating EAAT2. J Exp Med 2008; 205: 2473–81.
[74] Jarius S, Paul F, Franciotta D, Waters P, Zipp F, Hohlfeld R, et al. Mechanisms of disease: aquaporin-4 antibodies in neuromyelitis optica. Nat Clin Pract Neurol 2008; 4: 202–14.
[75] Cree B. Neuromyelitis optica: diagnosis, pathogenesis, and treatment. Curr Neurol Neurosci Rep 2008; 8: 427–33.
[76] Matsushita T, Matsuoka T, Isobe N, Kawano Y, Minohara M, Shi N, et al. Association of the HLA-DPB1*0501 allele with anti-aquaporin-4 antibody positivity in Japanese patients with idiopathic central nervous system demyelinating disorders. Tissue Antigens 2009; 73: 171–6.
[77] Poole BD, Templeton AK, Guthridge JM, Brown EJ, Harley JB, James JA. Aberrant Epstein-Barr viral infection in systemic lupus erythematosus. Autoimmun Rev 2009; 8: 337–42.
[78] Jacob A, Weinshenker BG, Violich I, McLinskey N, Krupp L, Fox RJ, et al. Treatment of neuromyelitis optica with rituximab: retrospective analysis of 25 patients. Arch Neurol 2008; 65: 1443–8.
[79] Waters P, Jarius S, Littleton E, Leite MI, Jacob S, Gray B, et al. Aquaporin-4 antibodies in neuromyelitis optica and longitudinally extensive transverse myelitis. Arch Neurol 2008; 65: 913–9.
[80] Waters P, Vincent A. Detection of anti-aquaporin-4 antibodies in neuromyelitis optica: current status of the assays. Int MS J 2008; 15: 99–105.
[81] Hayakawa S, Mori M, Okuta A, Kamegawa A, Fujiyoshi Y, Yoshiyama Y, et al. Neuromyelitis optica and anti-aquaporin-4 antibodies measured by an enzyme-linked immunosorbent assay. J Neuroimmunol 2008; 196: 181–7.
[82] Jarius S, boul-Enein F, Waters P, Kuenz B, Hauser A, Berger T, et al. Antibody to aquaporin-4 in the long-term course of neuromyelitis optica. Brain 2008; 131: 3072–80.
[83] Takahashi T, Fujihara K, Nakashima I, Misu T, Miyazawa I, Nakamura M, et al. Establishment of a new sensitive assay for anti-human aquaporin-4 antibody in neuromyelitis optica. Tohoku J Exp Med 2006; 210: 307–13.
[84] Nicchia GP, Mastrototaro M, Rossi A, Pisani F, Tortorella C, Ruggieri M, et al. Aquaporin-4 orthogonal arrays of particles are the target for neuromyelitis optica autoantibodies. Glia 2009; Feb 19 (in press).

[85] Tani T, Sakimura K, Tsujita M, Nakada T, Tanaka M, Nishizawa M, et al. Identification of binding sites for anti-aquaporin 4 antibodies in patients with neuromyelitis optica. J Neuroimmunol 2009; 211: 110–3.
[86] Selak S, Mahler M, Miyachi K, Fritzler ML, Fritzler MJ. Identification of the B-cell epitopes of the early endosome antigen 1 (EEA1). Clin Immunol 2003; 109: 154–64.
[87] Fukuda T, Shiraishi H, Nakamura T, Tanaka K, Nakamura H, Tsujino A, et al. Efficacy of tacrolimus in Sjogren's syndrome-associated CNS disease with aquaporin-4 autoantibodies. J Neurol 2009, in press.
[88] Sofat N, Venables PJ. Is Sjogren myelopathy Devic disease? Ann Rheum Dis 2008; 67: 730–1.
[89] Birnbaum J, Kerr D. Devic's syndrome in a woman with systemic lupus erythematosus: diagnostic and therapeutic implications of testing for the neuromyelitis optica IgG autoantibody. Arthritis Rheum 2007; 57: 347–51.
[90] Mehta LR, Samuelsson MK, Kleiner AK, Goodman AD, Anolik JH, Looney RJ, et al. Neuromyelitis optica spectrum disorder in a patient with systemic lupus erythematosus and anti-phospholipid antibody syndrome. Mult Scler 2008; 14: 425–7.
[91] Kay CS, Scola RH, Lorenzoni PJ, Jarius S, Arruda WO, Werneck LC. NMO-IgG positive neuromyelitis optica in a patient with myasthenia gravis but no thymectomy. J Neurol Sci 2008; 275: 148–50.
[92] McKeon A, Lennon VA, Jacob A, Matiello M, Lucchinetti CF, Kale N, et al. Coexistence of myasthenia gravis and serological markers of neurological autoimmunity in neuromyelitis optica. Muscle Nerve 2009; 39: 87–90.
[93] Jarius S, Jacob S, Waters P, Jacob A, Littleton E, Vincent A. Neuromyelitis optica in patients with gluten sensitivity associated with antibodies to aquaporin-4. J Neurol Neurosurg Psychiatry 2008; 79: 1084.
[94] Heerlein K, Jarius S, Jacobi C, Rohde S, Storch-Hagenlocher B, Wildemann B. Aquaporin-4 antibody positive longitudinally extensive transverse myelitis following varicella zoster infection. J Neurol Sci 2009; 276: 184–6.
[95] Kovacs B, Lafferty TL, Brent LH, Dehoratius RJ. Transverse myelopathy in systemic lupus erythematosus: an analysis of 14 cases and review of the literature. Ann Rheum Dis 2000; 59: 120–4.
[96] Vincent TL, Richardson MP, Mackworth-Young CG, Hawke SH, Venables PJ. Sjogren's syndrome-associated myelopathy: response to immunosuppressive treatment. Am J Med 2003; 114: 145–8.
[97] Johnstone RM. Exosomes biological significance: A concise review. Blood Cells Mol Dis 2006; 36: 315–21.
[98] Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics 2009; 6: 267–83.
[99] McLellan A. Exosome release by primary B cells. Crit Rev Immunol 2009; 29: 203–17.

Abbreviations

Aab, autoantibodies; **ALBIA**, addressable laser bead immunoassay; **AGA**, anti-Golgi antibodies; **ANA**, antinuclear antibody; **AQP4**, aquaporin 4; **CCP**, cyclic citrullinated peptide; **CENP**, centromere protein; **dsDNA**, double stranded DNA; **Elisa**, enzyme linked immunoassay; **IIF,** indirect immunofluorescence; **LIA**, line immunoassay; **MS**, multiple sclerosis; **NHL**, non-Hodgkin's lymphoma; **NMO**, neuromyelitis optica; **OSAD**, organ specific autoimmune diseases; **RA**, rheumatoid arthritis; **RIA**, radioimmunoassay; **RNP**, ribonucleoprotein; **SARD**, systemic autoimmune rheumatic diseases; **SjS**, Sjögren's syndrome; **SLE**, systemic lupus erythematosus; **SSc**, systemic sclerosis; **Sm**, Smith antigen.

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