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REVIEW ARTICLE

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Human autoantibodies as reagents in biomedical research

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Abstract Autoantibodies are typically associated with autoimmune diseases. In some instances the association of specific autoantibodies to a specific autoimmune disease have made their detection invaluable in clinical diagnosis. However, certain autoantibodies show no specific disease association and therefore have limited clinical utility. Nevertheless, autoantibodies are powerful tools for identification, characterization, and functional studies of their cognate antoantigens. In addition, the study of autoantibodies and their cognate autoantigens in human disease and in experimental animal models can provide valuable insight into disease mechanisms and the factors that ameliorate or reverse disease. This review will focus on three specific sets of autoantibodies, which were initially selected for investigation purely on the basis of their novel patterns of reactivity. These were observed when they were applied to a diagnostic HEp-2 test slide for antinuclear antibody detection by indirect immunofluorescence. The target autoantigens were identified as the *trans*-Golgi network protein GOLGA4 (Golgin 245 or p230), the early endosome antigen-1 (EEA1) and a yet to be identified and fully characterized phosphoepitope(s) restricted to chromosomal arms of condensed mitotic/meiotic chromosomes (MCA1). This laboratory has exploited sera which are reactive to these autoantigens for their identification and characterization, and in functional studies. This review highlights the uses of autoantibodies that may have limited diagnostic or prognostic utility, but are nonetheless novel reagents in the prosecution of molecular cell biology.

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Introduction

The origin of autoantibodies remains poorly understood.[1,2] Pathological autoantibodies tend to belong to the IgG immunoglobulin class, have high binding affinities, and occur in elevated titers. In contrast, natural autoantibodies that occur in healthy individuals usually have low titers, poor affinity for their cognate autoantigen(s), and mainly belong to the IgM class, although some natural IgGs and IgAs have also been described.[3,4]

Some autoantibodies are useful for the clinical diagnosis and prognosis of human autoimmune diseases, the study of autoimmune pathology and etiology, and investigations of the function of the targeted molecule. The association of specific autoantibodies with organ-specific and systemic autoimmune disease is well documented and reviewed.[5,6] However, not all autoantibodies have a clinical utility. Nevertheless, the study of autoantibodies and their cognate autoantigens can provide valuable insight into disease mechanisms and contribute to the development of novel technologies that ameliorate or reverse disease.

The unique property of antibodies is their ability to bind specifically to the target molecule. The affinity of antibodies for their cognate targets makes them ideal molecular probes for the characterization and identification of the target molecule. For biological studies, autoantibodies have tended to be superior to antibodies raised against self or non-self antigens. This is due in part to the nature of autoantibodies, which most often recognize epitopes that are highly conserved in evolution, allowing their exploitation as molecular probes in a variety of animal model systems.[7–9] The autoepitopes, which are conserved in nature across the species, often form part of the critical functional domains of the target molecule. This property of autoantibodies can often be employed in biological assays addressing the

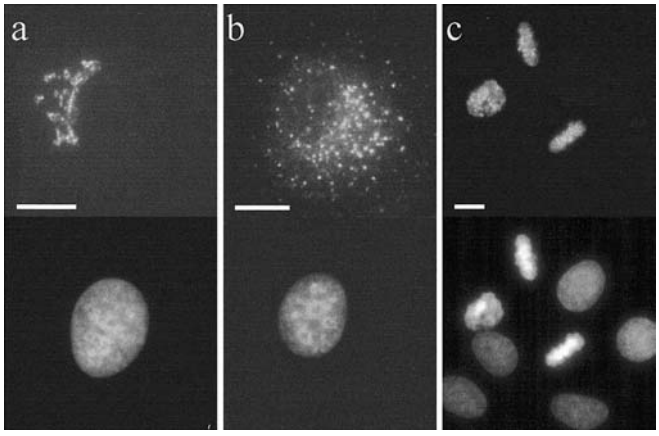

Fig. 1. Indirect immunofluorescence on fixed human HEp-2 cells with human autoimmune sera with reactivity to **a** p230, **b** EEA1, and **c** MCA1. Bound human autoantibodies were detected with goat antihuman immunoglobulin conjugated to rhodamine. Slides were counter-stained with Hoechst 33342 (DNA dye). *Top*, autoimmune sera; *bottom*, DNA dye. *Bar* 10 μm

mechanisms of the action and function of the target molecule in model systems and in situ.

Here, we review a set of autoantibodies that were selected for investigation purely on the basis of their novel patterns of reactivity observed on diagnostic HEp-2 test slides. The target molecules discussed will include the *trans*-Golgi network protein GOLGA4, early endosome antigen-1 (EEA1), and a yet to be identified and fully characterized protein with autophosphoepitope(s) whose distribution appears to be restricted to chromosomal arms of condensed mitotic/meiotic chromosomes (MCA1) (Fig. 1). Although this set of autoantibodies has not demonstrated particular diagnostic utility, as molecular probes they have contributed greatly to our current knowledge of the target molecule through its identification and characterization, and in functional studies.

Golgi autoantigens: GOLGA4 (Golgin 245 or p230)

The Golgi apparatus is the central organelle in the secretory pathway which is responsible for post-translational modification of proteins synthesized in the endoplasmic reticulum, and for sorting and delivering these proteins to their final destination. It is located in a juxtanuclear position near the microtubule organizing center of the cell. Autoantibodies directed against the Golgi apparatus were first described in 1982 in a patient with Sjögren's syndrome and lymphoma,[10] and have since been reported during routine examinations of patients' sera and as a result of several systematic surveys.[11] Golgi autoantigens ranging in size from 35 to 400 kDa, and their disease associations have been reported[12–14] (Table 1). Many of these autoantibodies have been utilized for the molecular identification and characterization of the cognate Golgi autoantigens[16–28] (Table 2). One of these Golgi autoantigens, GOLGA4, which was identified and characterized in this laboratory using human autoantibodies, will be the focus of our discussion of the Golgi reactive autoantibodies.

Table 1. Diseases associated with anti-Golgi antibodies (AGA)

Diseases reported to have high frequencies of AGA[a]	%
Sjögren's syndrome	40.5
Viral infections	
Cytomegalovirus	35.5
Epstein–Barr virus (infectious mononucleosis)	33
HIV-1	36
Rubeola	19.5
Normal control	10
Diseases associated with 50 patients with AGA[b]	**%**
Primary and secondary Sjögren's syndrome	50
Other rheumatic disease	30
Viral hepatitis/HIV	10
Carcinomas	5
Neurological disorders	3
Other	2

[a] Rattner and Fritzler[15]
[b] Unpublished data of 50 sera with AGA selected at random (supplied by Prof. M. Fritzler, University of Calgary, Canada)

Human autoantibodies reactive to GOLGA4 were first reported in high titer, in combination with lower-titer antinuclear autoantibodies, in a patient with Sjögren's syndrome.[29] Three additional sera, one with concordant nuclear reactivity, from patients with Sjögren's syndrome have also been reported.[18,19,30] There have been no reports to date of systematic surveys for the identification of GOLGA4-reactive autoantibodies.

Fritlzer et al.[19] and Erlich et al.[18] used high-titer autoimmune sera in immunoscreening assays of UniZAP and λgt11 HeLa cell cDNA expression libraries, respectively, to identify cDNAs encoding GOLGA4. To obtain additional cDNA sequences, Fritzler et al.[19] used a 5′-RACE methodology, and Erlich et al.[18] immunoscreened a λ ZAP hepatoma cDNA library.

p230,[18] also named golgin-245,[19] is an autoantigen of approximately 261 kDa. The gene encoding this autoantigen has been named *GOLGA4* (after Golgi autoantigen, golgin subfamily a, 4) by the Human Genome Nomenclature Committee (HUGO). Electron microscopy studies, using affinity-purified autoantibodies, have demonstrated that GOLGA4 is a peripheral membrane protein, localized to the cytoplasmic face of the *trans*-Golgi network (TGN) and on vesicles budding from the TGN.[18,29,31] GOLGA4 cycles between cytosol and membranes of the TGN and nonclathrin-coated budding vesicles in a G protein-regulated manner, dissociating from membranes following brefeldin-A treatment (albeit with delayed kinetics compared with conventional coat proteins), and recruited to membranes by GTPγS and AlF4 treatments.[31]

GOLGA4 consists of three domains: a flexible proline-rich amino terminal domain, a rigid central α-helical coiled-coil domain, and a flexible carboxyl terminal domain (Fig. 2). The carboxyl terminal domain harbors a 42-amino acid sequence (the Golgi localization domain (GLD))[33] which is sufficient for GOLGA4 localization to the Golgi. A number of other Golgi proteins utilize sequence motifs related to

Table 2. Molecularly identified Golgi-associated autoantigens

Gene[a]	Other published names	Reference seq.[b]	Reference
GOLGA1[c]	Golgin-97	NM-002077	Griffith et al.[24]
GOLGA2	Golgin-95, GM130	NM-004486	Fritzler et al.[20]
GOLGA3	Golgin-160	NM-005895	Fritzler et al.[20]
GOLGA4	p230, GOLG, golgin-245, golgin-240, GCP2	NM-002078	Erlich et al.[18]
GOLGA5[d]	rfg5, RET II, golgin-84, RFG5	NM-005113	Bascom et al.[25] Ishizaka et al.[27]
GOLGB1	gcp 372, GCP, giantin	NM-004487	Linstedt and Hauri[28]

[a] Approved UCL/HGNC/HUGO human gene nomenclature database symbol
[b] Reference sequence mRNA NCBI locus identifier
[c] GOLGA, golgi autoantigen, golgin subfamily a
[d] GOLGA5 has not been reported to be associated with autoantibodies, but is included here because of structural and sequence similarities

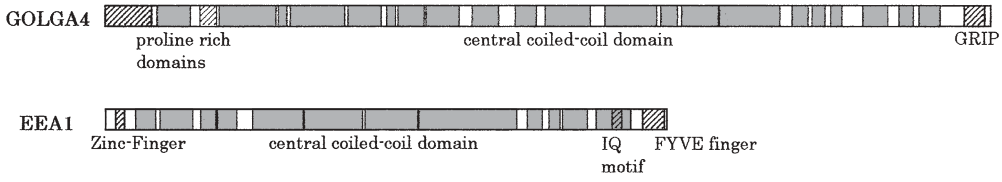

Fig. 2. Schematic representation of GOLGA4 and EEA1. Both molecules can generally be described as consisting of short amino and carboxyl terminal domains linked by a larger central α-helical coiled-coil domain. Predicted coiled-coil regions are shaded *gray*.[32] The amino terminal proline-rich domains and the grip domain of GOLGA4 are *hatched* and indicated, as are the zinc fingers and IQ motif of EEA1. See text for a description of splice variants of GOLGA4. Note that no splice variants have been reported for EEA1. In the case of MCA1, the molecular identity of this autoantigen is yet to be resolved

the GOLGA4 GLD for localization to the Golgi.[34] The GLD domain is now widely referred to as the GRIP domain[35] after the four proteins in which it is found, and about which something is known (golgin-97, RanBP2α, Imh1p, and p230/GOLGA4).

A number of splice variants of GOLGA4 have been reported.[18,30] Erlich et al.[18] identified cDNAs encoding the carboxyl terminal domain of GOLGA4 with varying sequence, indicating the presence of two splice variants. One seven-amino acid insertion and another nine-amino acid insertion, that results in an alternative stop codon in place of an open reading frame termination, were identified in the carboxyl terminal region, where the GRIP domain exists. In the amino terminal domain of GOLGA4, Tsukada et al.[30] reported a 22-amino acid insertion in the first proline rich sequence, always coupled to a 10-amino acid deletion, and referred to as the YT variant, in contrast to the original sequence of the RE variant. Splice variants in the carboxyl domain of GOLGA4 can occur independently of each other, and are also independent of the amino terminal splice variant(s). To date, no function(s) has been ascribed to the splice variants.

There are two conflicting reports ascribing the chromosomal localization of the GOLGA4 gene. Erlich et al.[18] utilized in situ hybridization to human chromosomes to localize GOLGA4 to chromosome 6 (p12–22). In contrast, specific tagged sequences demonstrate that GOLGA4 is localized to chromosome 3 (p22–p21.3) (human genome mapping program).

The function of GOLGA4 is not known. The association of p230 with nonclathrin-coated TGN budding vesicles suggests a role in protein trafficking from the TGN.

Cytoplasmic vesicle autoantigens: EEA1

The most prominent cytoplasmic structure is the Golgi apparatus, whose constituents we have discussed as targets for autoantibodies. Other cytoplasmic structures, particularly those that are membrane-bound and vesicle-like in appearance, have also been targets for autoantibodies. Many autoantibodies reactive to the mitochondria (AMA) have been reported and reviewed elsewhere.[36–38] Excluding AMA from the present discussion, autoantibodies to cytoplasmic vesicle-like structures occur infrequently. The prevalence of this staining pattern has been reported to be less than 0.1%.[39,40] Other studies have reported a higher prevalence; 3.8% in a survey of female blood donors,[41] and 10.4% of "normal" subjects.[42] However, these were predominantly low titer. To date, apart from the mitochondria autoantibody targets, only four autoantigen targets associated with cytoplasmic vesicle-like structures have been identified[39,43–45] (Table 3). One of these targets, early endosome antigen 1 (EEA1), is the focus of our discussion of this group of autoantibodies.

Autoantibodies reactive to EEA1 were first identified in a high titer autoimmune serum collected from a patient with

Table 3. Molecularly identified vesicle-associated autoantigens[a]

Gene	Other published names	Reference seq.	Reference
EEA1	Early endosome associated protein	NM-003566	Mu et al.[43]
IGF2R	Insulin-like growth factor 2 receptor; CI-MPR[b]	NM-000876	Tarrago et al.[39] Oshima et al.[46]
LBPA[c]	Lysobisphosphatidic acid	–	Galve de Rochemonteix et al.[44]
TNRC6[d]	GW182, EDIE, KIAA1460	XM 047123	Eystathioy et al.[45]

[a] See also Table 2 footnotes
[b] Cation-independent mannose 6-phosphate receptor
[c] Target is not a protein, but a lysobisphosphatidic acid, localized to the internal membranes of late endosomes
[d] Trinucleotide repeat containing 6

subacute cutaneous lupus erythematosus.[43] We have also detected autoantibodies reactive to EEA1 in 13% of sera with cytoplasmic vesicle-like reactivity (5/38).[40] The patients with EEA1-reactive autoantibodies were all female and over 60 years of age, most had generalized rheumatological clinical features, and one had Sjögren's syndrome. Selak et al.[47] selected sera on the basis of cytoplasmic reactivity, and reported that 8 of 36 (22%) patients, ranging in ages from 48 to 86, four of whom were male, showed reactivity with EEA1. Three-quarters of these patients presented with a diverse spectrum of neurological disease.

Previously, we reported on a high titer autoimmune serum from a patient with subacute cutaneous lupus erythematosus that reacted with a 162-kDa protein colocalized with rab5 and internalized transferrin receptor.[43] We named this antigen EEA1 (after early endosome antigen-1). EEA1 localizes to the cytoplasmic face of early endosomes and is present in the cytosol. Using this autoimmune serum to screen human HeLa and hepatoma cDNA expression libraries, we were able to construct a 5-kb cDNA generated from several truncated overlapping cDNAs. The 5-kb cDNA contains one large open reading frame of 4233 nucleotides encoding a protein of 1410 amino acids. Northern blot analysis indicates that the full-length EEA1 mRNA is approximately 9 kb.

EEA1 consists of extensive coiled-coil regions and contains a calmodulin-binding IQ motif, while both the amino and carboxyl terminal regions contain sequence motifs reminiscent of zinc fingers (see Fig. 2).[43] We have demonstrated in vitro that the C-terminal zinc finger motif constitutes a genuine zinc binding domain.[48] This cysteine-rich motif is shared with Vps27, Fab1, and Vac1: yeast proteins implicated in membrane traffic. Accordingly, we have named this zinc binding domain "FYVE finger" (based on the first letters of the four proteins we had identified as containing this motif).

EEA1 functions as a tethering protein for early endosome fusion. The FYVE domain of EEA1 has been implicated in a number of interactions, including phosphatidylinositol(3)-phosphate,[49,50] Rab5-GTP,[51,52] syntaxin 6,[53] and syntaxin 13.[54] The IQ motif of EEA1, which is juxtaposed to the C-terminal FYVE finger, has been shown to interact with calmodulin.[55] The presence of both the FYVE finger domain and the IQ motif are required for EEA1 binding to early endosomes.[48] The multiplicity of interaction partners raises the prospect of cooperation and/or competition for binding at the FYVE finger domain by both lipid and protein partners.

Mitotic chromatin autoantigens: MCA1

The mitotic apparatus is restricted to the M phase of the cell cycle. Autoantibodies to this structure have been classified into three broad groups based on reactivity to the mitotic spindle apparatus, chromosomes, and centrosomes.[15,56,57] Some of the autoantigen targets associated with the mitotic apparatus are reactive only in a limited phase of the cell cycle.[58–64] This review focuses on the group of autoantibodies that recognize M-phase condensed mitotic chromosomes, which display no apparent reactivity to chromosomes in other stages of the cell cycle (Table 4). This group of mitotic apparatus autoantibodies are currently the least well characterized, with the molecular identity of the target autoantigens not having been resolved. In the case of anti-"dividing cell antigen" (DCA) autoantibodies, their presence in several defined autoimmune disease groupings has been investigated,[61] while the presence of anti-MCAs autoantibodies has not been investigated. The MCA autoantigens were first defined and characterized in this laboratory, and we will focus our discussion on MCA1, the best characterized of this autoantigen group.

Human autoantibodies reactive to MCA1, after mitotic chromosomal autoantigen 1 (previously reported as RMSA1),[65,66] were detected in a female patient with discoid lupus erythematosus and concurrent chronic lymphocytic leukaemia.[62,65] These autoantibodies occur infrequently; i.e., in around 0.005% of sera referred for ANA investigation.[62] However, MCA1 detection could be masked by other ANA reactivity that persists into the M phase.

Autoantibodies reactive to MCA1 display a speckled pattern of staining from early prophase to late anaphase along the arms of the chromosomes. MCA1 staining is not detected in the nucleus. The archetypal autoantibodies reactive to MCA1 are IgG_2 with κ light chains.[62] Traditional methods employing the MCA1 reactive autoantibodies, such as immunoscreening of expression libraries for the identification of cDNA clones encoding MCA1, and immu-

Table 4. Autoantigens with exclusive immunofluorescence staining of mitotic chromosome arms with no staining of interphase nuclei

Antigen[a]	Distinctive inhibitor of antibody–antigen interaction	Disease association[b]	Reference
DCA	Absorption of autoimmune serum to histones H2A and H2B extract	Systemic lupus erythematosus	Blaschek et al.[61]
MCA1	Pretreatment of HEp-2 test slide with a serine–threonine phosphatase	Discoid lupus erythematosus	Gitlits et al.[62]
MCA2	Pretreatment of HEp-2 test slide with a serine–threonine phosphatase	Sjögren's syndrome	Gitlits et al.[62]
MCA3	Pretreatment of HEp-2 test slide with a tyrosine phosphatase	Polymyalgia rheumatica	Gitlits et al.[62]

[a] DCA, dividing cell antigen; MCA, mitotic chromosomal autoantigen
[b] Disease association is based on the first patient reported with said autoantibodies. In the case of DCA, a limited survey found 10/169 (~6%) systemic lupus erythematosus, 0/75 rheumatoid arthritis, 0/60 scleroderma, 0/48 Sjögren's syndrome, and 1/39 (~3%) Raynaud's phenomenon patients with reactivity to DCA, compared with 0/304 control subjects[61]

noprecipitation or immunoblotting of MCA1 from subcellular fractions for protein purification and sequence determination, have proven refractive for revealing the molecular identity of the autoantigen. Nevertheless, autoantibodies reactive to MCA1 have contributed to the characterization of the biochemical properties of the autoantigen and its essential requirements for mitosis to proceed. The autoantigen is protein in nature, and its detection is not ablated by RNase or DNase treatments, but is affected by salt and acid treatments of fixed mitotic cells.[62] The autoreactive epitope(s), detected by the archetypal autoantibodies to MCA1, may be phosphorylated, or the epitope(s) conformation may be altered by the phosphorylation status of the autoantigen or associated partners. The evidence for this assertion is that detection of MCA1 is impossible after treatments with λ-PPase (with activity to phosphoserine/threonine/tyrosine residues) and PP1 (with activity to phosphoserine/threonine residues), but it is insensitive to YOP (with activity to phosphotyrosine residues). It has been postulated that MCA1 may play a role in sister chromatid separation. This assertion is supported by the observation that microinjection of autoantibodies reactive to MCA1 into the cytoplasm of HeLa cells in interphase led to mitotic arrest during the next M phase at metaphase, with no apparent change in the morphology of either the chromosomes or the mitotic spindle.[62]

It is not uncommon for autoimmune sera to harbor autoantibodies reactive to more than one autoantigen. In the case of the MCA1-reactive autoimmune sera, we have previously demonstrated the presence of other autoantibodies with reactivity to glycolytic enzyme enolase,[9] the synaptic vesicle coat protein synapsin 1,[67] and another still unidentified brain-specific 33 kDa autoantigen.

Conclusion

As a consequence of the Human Genome Project, we are now able to predict the complete set of genes encoding the proteins required for the development of the human form. However, the timing of the expression of these genes, the tissues and organs in which they are expressed and their spatial (extracellular and subcellular) distribution, the interacting partners, and function of the majority of these proteins are yet to be fully elucidated. The properties of human autoantibodies discussed here make them ideal tools for biochemical, molecular, and functional characterization of their cognate targets. Autoantibodies utilized in such investigations are superior to their cousins, which are generated by various immunization strategies, as they often include antibodies with reactivity to epitopes conserved in evolution, and frequently bind to "active" sites of the molecule. In the future, as we develop a clear unequivocal understanding of the mechanisms of the induction of autoimmunity, not only may we be able to induce autoantibody production in animal models to an antigen of choice for use as a research tool, but we will also be able to apply this knowledge to eradicating human autoimmune disease.

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