

Molecular Characterization of Virus-induced Autoantibody Responses

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Abstract

Here we present a comprehensive molecular mapping of virus-induced autoimmune B cell responses obtained by serological identification of antigens by recombinant expression cloning analysis. Immunoscreening of cDNA expression libraries of various organs (lung, liver, and spleen) using sera from mice infected with cytopathic (vaccinia virus [VV]) or noncytopathic (lymphocytic choriomeningitis virus [LCMV]) viruses revealed a broad specificity of the elicited autoantibody response. Interestingly, the majority of the identified autoantigens have been previously described as autoantigens in humans. We found that induction of virus-induced autoantibodies of the immunoglobulin G class largely depends on the CD40–CD40L-mediated interaction between T and B cells. Furthermore, antibody titers against a number of autoantigens were comparable to the concomitantly induced antiviral antibody response. Comparison of serum reactivity against a selected panel of autoantigens after infection with VV, LCMV, or vesicular stomatitis virus showed that the different virus infections triggered distinct autoantibody responses, suggesting that virus infections may leave specific “autoantibody fingerprints” in the infected host.

Key words: autoantibodies • tumor immunity • virus-induced immunopathology • SEREX

Introduction

Infections with viruses as well as other pathogens lead to antigen-specific induction of T and B lymphocytes. However, these usually protective responses are often accompanied by the appearance of autoantibodies to self-antigens of the host (1). Autoimmune diseases are usually associated with autoantibodies directed against cells of the target tissue (2). The majority of these antibody responses probably do not cause direct tissue injury. However, certain conditions may favor the appearance of autoantibodies that possess a high pathogenic potential (3, 4).

The concept that viral infections contribute to the development of autoimmune diseases is supported by a wide range of experimental and clinical observations. For example, infections with pancreatropic viruses such as mumps virus

or Coxsackie virus B often precede the onset of insulin-dependent diabetes mellitus (5). Virus infections that are associated with hypergammaglobulinemia such as Epstein-Barr virus, generally elicit broadly reactive autoantibody responses with serum antibodies to DNA, cytoskeletal proteins, or thyroglobulin (6). Furthermore, autoimmune B cell responses most likely contribute to the extrahepatic manifestations, such as mixed cryoglobulinemia, glomerulonephritis, and vasculitis, of hepatitis B and C virus infection (7, 8).

Several mechanisms may account for the generation of autoantibodies in the cause of viral infections. First, antiviral antibody responses may cross-react with self-determinants due to molecular mimicry based on homologies in the amino acid sequence (9). Second, release of self-antigens

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Abbreviations used in this paper: COL13A1, procollagen XIII; GOLGB1, macroglobulin; LCMV, lymphocytic choriomeningitis virus; MYH6, myosin heavy chain α ; ROCK, Rho-associated kinase; SEREX, serological identification of antigens by recombinant expression cloning; VSV, vesicular stomatitis virus; VV, vaccinia virus.

through direct cytolytic activity of the infectious agent or by subsequent immune responses directed against infected cells may precipitate bona fide autoimmunity against antigens of the target tissue. Furthermore, virus infection-associated activation of self-reactive “bystander” T and B lymphocytes may exacerbate autoimmune reactions (10).

The insights in the pathogenesis of antibody-mediated autoimmunity have been gained largely by experimental studies using defined model antigens. However, many aspects of virus-induced autoimmunity remain elusive because the complexity of the virus-induced autoantibody repertoire is still not completely understood. A particular drawback is that the molecular nature of autoantigens as potential drivers of the progressive autoimmune disease has not yet been systematically dissected.

Detection of human autoantigens has been based on microscopical detection of autoantibodies reacting with an affected tissue. Subsequent biochemical analyses, frequently involving immunoprecipitation and immunoblotting, facilitated molecular identification of the autoantigen. Identification and molecular characterization of self-antigens eliciting linked T and B cell responses has been fostered by λ phage-based expression screening systems (11). For example, the serological identification of antigens by recombinant expression cloning (SEREX) approach, originally developed for the identification of tumor antigens, facilitates the systematic and unbiased dissection of the autoreactive B cell repertoire (12–14). For several tumor antigens identified by SEREX, strong concomitant T helper cell and CTL responses have been observed in cancer patients, indicating that this approach is a convenient tool to analyze complex immune responses against self-antigens (15–18). Furthermore, the suitability of the SEREX approach to define autoantigens associated with human autoimmune diseases such as systemic lupus erythematosus and systemic sclerosis has been shown recently (19–21).

In this study, we used the SEREX technique for molecular mapping of the autoantibody repertoire in mice after infection with cytopathic and noncytopathic viruses. Our analysis revealed an intriguingly broad polyclonal autoimmune B cell response against mainly intracellular proteins, including a large number of “SEREX antigens” previously identified with human sera.

Materials and Methods

Viruses and Mice. Lymphocytic choriomeningitis virus (LCMV)-WE was originally obtained from F. Lehmann-Grube (University of Hamburg, Hamburg, Germany) and was propagated on L929 cells. Recombinant vaccinia virus (VV) expressing vesicular stomatitis virus (VSV) glycoprotein (VV-INDG) was provided by B. Moss (National Institutes of Health, Bethesda, MD) and grown on BSC 40 cells. VSV Indiana (VSV-IND; Mudd-Summers isolate) was originally obtained from D. Kolakofsky (University of Geneva, Geneva, Switzerland). VSV was propagated on baby hamster kidney 21 cells. Mice were infected with either 200 PFU LCMV-WE (low dose), 2×10^5 PFU LCMV-WE (high dose), 2×10^6 PFU VV-INDG, or 2×10^6 PFU VSV-IND via i.v. in-

jection. In vivo depletion of CD4 T cells was performed by injection of rat monoclonal antibody (YTS 191.6) on days -3 and -1 before infection (22). C57BL/6, CD40L-deficient (23), and IgM-deficient (24) mice were obtained from the Institut fuer Labortierkunde (University of Zurich, Zurich, Switzerland). All animals were kept under specific pathogen-free conditions. Experiments were performed with age- (8–12 wk) and sex-matched animals.

Construction of cDNA Expression Libraries. If cDNA expression libraries are derived from B cell-infiltrated tissues, SEREX immunoscreening might be seriously hampered by direct binding of the second antibody to recombinantly expressed Ig transcripts that may represent the majority of “reactive” clones. These “false positive” clones can be identified in a prescreening procedure whereby the membranes are first incubated with enzyme-conjugated anti-human IgG antibody (25). To exclude the detection of false positive clones in our SEREX analysis of virus-induced murine autoimmune B cell responses, cDNA libraries were generated from tissues of B cell-deficient mice. Organs from perfused B6- μ MT mice were shock frozen in liquid nitrogen and stored at -80°C . Total RNA was isolated from 0.5–1 g of tissue using the method described by Chomczynski and Sacchi (26). Poly(A)⁺ RNA was isolated and used to construct cDNA libraries using ZAP Express cDNA synthesis kit and ZAP Express Gigapack III Gold Cloning kit (Stratagene) as described previously (27). The libraries consisted of $2\text{--}10 \times 10^6$ primary recombinants. $2\text{--}10 \times 10^5$ clones from each library were screened according to established SEREX protocols.

Immunoscreening. Serum was collected from mice at the indicated time points after immunization and stored at -20°C . Pooled serum from 10 mice was preabsorbed three times against bacteria lysed by nonrecombinant ZAP Express phages. The preabsorbed serum mix was diluted to a final concentration of 1:500 in $1 \times \text{TBS}$, 1% dry milk, and 0.01% Na-azide.

SEREX immunoscreening has been described elsewhere in detail (28). In brief, *Escherichia coli* XL1 blue MRF' cells were transfected with the different expression libraries and plated to an approximate density of 5,000 PFU/plate. Protein expression was induced with 0.8 mM isopropyl- β -D-thiogalactoside (IPTG; Roche Diagnostics). Recombinant proteins were blotted onto Protran nitrocellulose membranes (Schleicher-Schüll). After washing in TBST (8.8 g NaCl, 1.4 g Tris, 6 g Tris-HCl, 5 ml Tween 20) and blocking with 5% (wt/vol) milk powder in TBS, membranes were incubated with prediluted (1:500) serum for 12–15 h. This serum dilution has been chosen because preimmune sera from naive animals did not show a specific reactivity with single clones. Antigen-antibody complexes were detected with goat anti-mouse IgG antibody conjugated to alkaline phosphatase (Jackson ImmunoResearch Laboratories). Reactive plaques were visualized with 5-bromo-4-chloro-3-indolyl-phosphate-toluidin (BCIP; BIOMOL Research Laboratories, Inc.) and nitroblue-tetrazoliumchlorid (NBT; BIOMOL Research Laboratories, Inc.).

Characterization of Positive Clones. Serum-reactive phages were monoclonalized, isolated, and in vivo excised to plasmid form using the ExAssist Interference-Resistant Helper Phage according to the manufacturer's instructions (Stratagene). Plasmid DNA was isolated using commercially available kits (QIAGEN). The lengths of DNA inserts were determined after double EcoRI and XhoI restriction endonuclease (Roche Diagnostics) digestion and size fractionation in standard TAE agarose gel electrophoresis. Sequencing was performed using the ABI Prism Cycle Sequencing kit (PerkinElmer) and run on an automated ABI Prism

Table I. Summary of the Immunoscreening with Sera from VV- and LCMV-infected Mice

Library ^a	Anti-VV sera ^b			Anti-LCMV sera ^b		
	Screened clones	Reactive clones	Different antigens ^c	Screened clones	Reactive clones	Different antigens
Mixed	7 × 10 ⁵	20	8	2 × 10 ⁵	4	3
Lung/VV	7 × 10 ⁵	42	14	2 × 10 ⁵	4	3
Ovary/VV	10 × 10 ⁵	104	35	4 × 10 ⁵	18	10

^amRNA for the generation of cDNA expression libraries was derived from various tissues of B cell-deficient mice: Mixed, 50% spleen, 30% liver, 20% lung; Lung/VV, VV-infected lung tissue on day 5 after infection; Ovary/VV, VV-infected ovary tissue on day 5 after infection.

^bC57BL/6 mice infected with 2 × 10⁶ PFU VV-INDG or 200 PFU LCMV-WE were bled on days 14 and 21 after infection. Pooled sera from 10 mice were used for immunoscreening.

^cReactive clones representing the same antigen were grouped and scored as one hit because abundant antigens were detected multiple times.

DNA sequencer. For sequence analysis, alignments with GenBank database were performed using the National Center for Biotechnology Information (NCBI) BLASTN and BLASTX algorithms to identify identities and homologies of genes. Human orthologues were identified by accessing the Homologene Database through LocusLink (NCBI). In selected cases, orthologues were confirmed using Mouse Genome Informatics resources (<http://www.informatics.jax.org/searches>). Mouse genes for which no human orthologue is cataloged as well as genes for which human orthologues have a different HUGO-approved nomenclature were flagged (see Tables II and III). The Cancer Immunome database harboring clones identified by SEREX analysis of human tumors was accessed at <http://www2.licr.org/CancerImmunomeDB> and analyzed for representation of human orthologues of cloned mouse autoantigens.

Differential Serological Analysis and Autoantibody Titration. Four to six phages (1–3 × 10² PFU) encoding monoclonalized individual antigens were mixed with wild-type λ ZAP phage as a control, used to infect exponentially growing *E. coli* XL-1 Blue MRF⁺, and separately plated on one 15-cm Petri dish. After overnight incubation, phage lysates were transferred onto nitrocellulose membranes by plaque lift, blocked with 5% (wt/vol) milk powder in TBS, and incubated overnight with twofold serum dilutions starting at 1:500. Washing and visualization with NBT and BCIP was performed as described above. Development of all blots was stopped when control λ ZAP phages started to show reactivity with sera from naive mice. The sero reactivity determined in this end-point titration method using crude phage lysates correlates well with the reactivity seen in Western Blot analysis with purified recombinant proteins (see below, Fig. S2).

Online Supplemental Material. The reactivity of self- and VV antigens tested with immune versus preimmune sera is shown in Fig. S1. In Fig. S2, the reactivity of anti-VV sera against recombinant proteins was assessed using semiquantitative Western Blot analysis. Figs. S1 and S2 are available at <http://www.jem.org/cgi/content/full/jem.20040358/DC1>.

Results

Detection of Immunoreactive Clones and Preliminary Characterization. To achieve broad transcriptional representation, we generated one library from lung, spleen, and liver tissue. In addition, to assess whether detection of viral antigens is feasible using the SEREX method and to assess the impact of

viral infection on the profile of detected autoantigens, we constructed cDNA libraries from both VV-infected lungs and ovaries, determined as organs with high viral load. Viral titers on day 5 after infection were 2–4 × 10⁴ PFU per lung and 2–6 × 10⁶ PFU per ovary. The cDNA libraries were screened with pooled sera from C57BL/6 mice infected either with 2 × 10⁶ PFU VSV glycoprotein-recombinant VV or with 200 PFU LCMV-WE. Successful priming of antiviral B cell responses was assessed by VSV neutralization assays for VV-INDG and nucleoprotein-specific ELISA for LCMV. On the peak of the antiviral B cell response (days 14–21), sera were collected and used pooled at 1:500 dilutions for immunoscreening. Between 7 and 10 × 10⁵ clones from each library were screened and a total of 166 and 26 clones were found to react with sera from VV- or LCMV-infected mice, respectively (Table I). Sequence analysis and homology search revealed that these clones represent 40 (VV infection, Table II) and 14 (LCMV infection, Table III) different antigens. The three libraries derived from different tis-

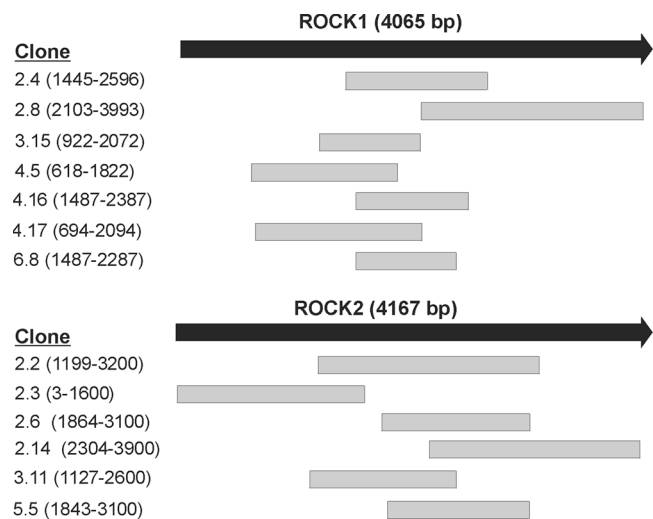


Figure 1. Alignment of selected cDNA clones encoding different regions in ROCK1 and ROCK2. Nucleotide position of the respective clone is indicated in parenthesis.

Table II. *Antigens Detected by IgG Antibodies from Sera of VV-infected Mice*

Homologue/identity ^a	Entry in SEREX database ^b	GenBank accession	Reactivity ^c	No. reactive clones in library		
				Lung/VV	Ovary/VV	Mixed
<i>Golgi- or ER-associated</i>						
GOLGB1	Orthologue	XM_148244	+++		1	
GOLGA4, Golgi autoantigen 4	Orthologue	NM_018748	++	3	1	3
4921537D05RIK, NY-REN-58	Orthologue	NM_029852	+	1	3	1
<i>Surface proteins</i>						
COL13A1	Homologues (many other collagens)	NM_007731	++			2
HMMR, hyaluronic acid binding protein	Orthologue	NM_013552	++		2	
<i>Cytoskeleton, structural elements</i>						
ROCK1	Orthologue	NM_009071	+++	16		1
ROCK2	Orthologue	NM_009072	++	6		
TPM1, α -tropomyosin 5b (rat)	Orthologue	NM_024427	++		1	
MYH6	Homologues MYH1, 10, 7	NM_010856	++	5	1	9
MYH4, myosin heavy polypeptide 4	Homologues MYH1, 10, 7	XM_126119	+	1	1	
2410197A17Rik, PPP1R12C (hu)	Homologues PPP1R12B, 14B, 14C	NM_029834	+		2	
LOC269661, KIAA0635 (hu)	Orthologue	XM_194221	++		2	
LMNA, Lamin C	Orthologue	NM_019390	+	1		
CYCLN2, CLIP-115	Orthologue	NM_009990	++		2	
DST, BPAG1 (hu)	Orthologue	NM_010081	+		2	1
4933432N21, TSGA10 (hu)	Homologue TSGA2	XM_136734	+		1	
RSN, CLIP-170	Orthologue	NM_010081	+	1	1	
<i>Heat shock proteins</i>						
DNAJA1, hsj2	Orthologue	NM_008298	++	4	2	3
DNAJC6, hsp40	Homologues DNAJA1, A2, B1	XM_283995	+		54	
HSPCA, heat shock protein 86	Orthologue	NM_010480	+	1	1	
<i>Nuclear factors and DNA binding</i>						
ATRX, X-linked nuclear protein	Orthologue	NM_009530	+++		1	
HOXB1, Homeobox B1	No	NM_008266	+++	1	1	
LEK1, CENPF (hu), centromere protein F	Orthologue	XM_129658	++		1	
EIF4GI (hu), translation factor	Homologue EIF4G2	NM_025816	++		2	

Table II. *Antigens Detected by IgG Antibodies from Sera of VV-infected Mice*

Homologue/identity ^a	Entry in SEREX database ^b	GenBank accession	Reactivity ^c	No. reactive clones in library		
				Lung/VV	Ovary/VV	Mixed
SF3B2, splicing factor 3b, subunit 2	No	NM_030109	+		3	
D6ERTD772E, TAX1BP1 (hu)	No	NM_025816	++		2	
<i>Miscellaneous</i>						
OFD1, oral-facial-digital syndrome 1	Orthologue	NM_177429	+		1	
FMO, flavin-containing monooxygenase 5	No	NM_010232	+	1		
NME3, nucleoside diphosphate kinase DR-nm23	Homologue NME1,2	NM_019730	++		2	
NY-REN-18 antigen	Orthologue	NM_016736	+		3	
FES, c-fes protooncogene	No	XM_133573	+		1	1
<i>Hypothetical</i>						
1810009B06RIK, KIAA1536 (hu)	Orthologue	NM_177385	++		1	
D13WSU177E, HSPC111 (hu)	No	XM_127366	++		1	
D10JHU81E, C21ORF33 (hu)	Orthologue	NM_138601	+	1	1	
D530005L17RIK, FLJ25636 (hu)	Orthologue	NM_177385	+	1	1	
<i>VV antigens</i>						
39-kD immunodominant antigen		S71673.1	++++		2	
P4b major core antigen		M27914	++++		1	
62-kD rifampicin-resistance gene		M15058, CDS21	++++		1	
A-type inclusion protein		M61187, CDS2	+++		2	

^aNCBI BLASTN and BLASTX algorithms were used to search GenBank database and obtain HUGO-approved nomenclature of clones under investigation. Mouse genes for which no human orthologues are catalogued as well as genes for which human orthologues have a different HUGO-approved nomenclature were flagged.

^bPresence of human orthologues or homologues in the SEREX database (<http://www2.licr.org/CancerImmunomeDB>).

^cSeroreactivity of the purified clones was assessed semiquantitatively by comparing the signal obtained with anti-VV serum against the 39-kD immunodominant antigen as reference signal (++++). The highest value is shown for antigens with multiple clones.

sues appeared complementary in dissecting as many entities of the autoantibody repertoire as possible. It is worth noting that the frequency of reactive clones was significantly higher in the screens with sera from mice infected with the cytopathic VV compared with those from mice infected with the noncytopathic LCMV.

Infection of mice with VV induced a broad and rather strong de novo autoimmune B cell response. The clones reacting with IgG from sera of VV-infected mice represented 36 autoantigens including Golgi-associated proteins, cytoskeletal and heat shock proteins, transcription factors, and proteins involved in DNA replication (Table II). LCMV infection also induced a broad autoimmune B cell response against proteins from various intracellular compartments (Table III). However, there was only a small overlap in the specificity, i.e., cardiac myosin heavy chain

α (MYH6) and hyaluronic acid binding protein were found in both immunoscreens (Tables II and III), suggesting that the two different viruses had elicited distinct patterns of autoreactivity on the B cell level. Most of the antigens were detected repeatedly. Independent clones were sequenced and aligned against the full-length cDNA sequence. As exemplified for Rho-associated kinase (ROCK)1 and ROCK2 in Fig. 1, not all clones were sharing one defined region of the respective protein. Analysis of the overlaps in all distinct transcripts from these two molecules that have been found in the screens with anti-VV serum provides evidence that the de novo autoimmune response is directed against at least two distinct epitopes in each antigen (Fig. 1).

Noticing that human orthologues of several of the identified autoantigens have been reported previously in

Table III. Antigenes Detected by SEREX Analysis Using Sera of LCMV-infected Mice

Homologue/identity ^a	Human orthologues in SEREX database ^b	GenBank accession	Reactivity ^c	No. reactive clones in library		
				Lung/VV	Ovary/VV	Mixed
<i>Golgi- or ER-associated</i>						
6030460N08RIK, TRIP11 (hu), thyroid hormone receptor interactor 11	Orthologue	XM_029327.1	++		1	
<i>Surface proteins</i>						
HMMR, hyaluronic acid binding protein	Orthologue	NM_013552.1	++		2	
LGALS8, galectin 8	Homologues LGALS1,4,9	NM_018886.1	+++		2	1
<i>Cytoskeleton, structural elements</i>						
MYH11, smooth muscle myosin heavy chain 11	Homologues MYH1, 10, 7, 9	NM_013607.1	++		2	
MYH6	Homologues MYH1, 10, 7, 9	M76600.1	+	2		
NIN, ninein	Orthologue	NM_008697.1	++		3	
CATNB, CTNNB1 (hu), β catenin	Homologues CTNNBLB1, A	NM_007614.1	+	1		
<i>Nuclear factors and DNA binding</i>						
HNRPAB, CArG box binding factor	Orthologue	L36663.1	++	1		
SATB1, special AT sequence binding protein 1	Orthologue	NM_009122.1	++		2	
<i>Miscellaneous</i>						
MDM2, mouse double minute clone 2	No	NM_010786	+		1	
TRIM2, tripartite motif protein 12	Orthologue	NM_030706			1	
RRBP1, ribosome receptor isoform	Orthologue	XM_194049	+		3	2
SDCCAG8, serological colon cancer antigen 8	Orthologue	AK017691.1	++		1	
DLAT, pyruvate dehydrogenase component E2	No	NM_145614	+			1

^aNCBI BLASTN and BLASTX algorithms were used to search GenBank database and obtain HUGO-approved nomenclature of clones under investigation. Mouse genes for which no human orthologues are catalogued as well as genes for which human orthologues have a different HUGO-approved nomenclature were flagged.

^bPresence of human orthologues of autoantigens under consideration in the SEREX database.

^cSeroreactivity of the purified clones was assessed semiquantitatively by comparing the signal obtained with anti-VV serum against the 39-kD immunodominant antigen as reference signal (++++). The highest value is shown for antigens with multiple clones.

the context of SEREX analysis of human malignancies, we systematically assessed the overlap. In fact, orthologues of 22 of 36 of VV-induced and 7 of 14 LCMV-induced autoantigens were also represented in the SEREX database. Orthologues of a total of 12 additional autoantigens were highly homologous and molecularly related to anti-

gens in the SEREX database (COLL13A1, MYH11, EIF4G1, NME3, etc.), suggesting cross-reactive epitopes. Thus, in summary, 83 and 79% of the proteins found in our screens using sera from VV- or LCMV-infected mice, respectively, represent orthologues of human cancer-associated autoantigens.

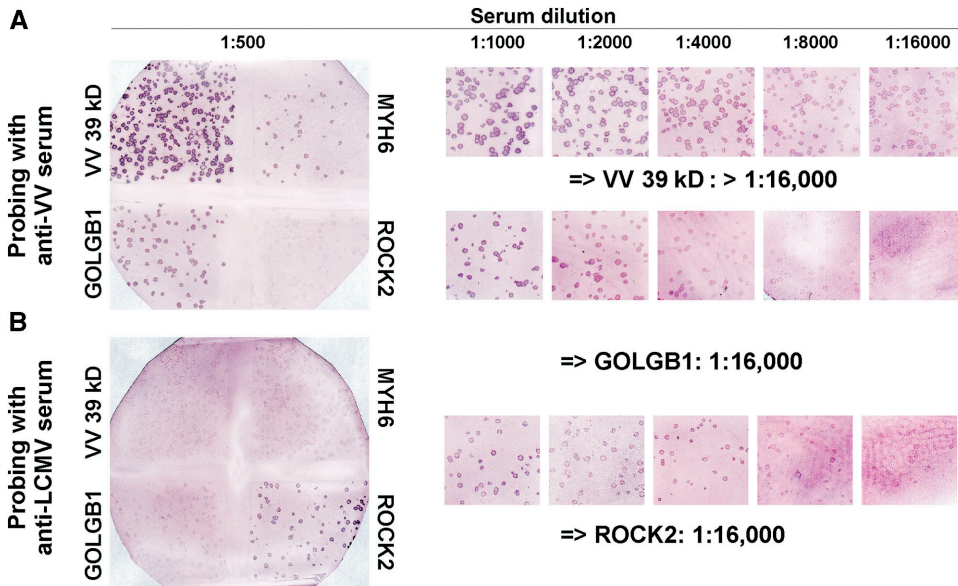


Figure 2. Rapid determination of seroreactivity pattern after viral infection. Phages from clones encoding for VV 39-kD immunodominant antigen, MYH6, GOLGB1, and ROCK2 were mixed with wild-type phage clones as negative control and used to transfect *E. coli*. After transfer onto nitrocellulose membranes, replica-plated phages were incubated with serial twofold dilutions of pooled serum from (A) mice infected with 2×10^6 PFU VV (day 14 after infection) or (B) mice infected with 2×10^5 PFU LCMV.

Differential Serological Analysis. To investigate the mechanisms of virus-induced autoimmune B cell responses in more detail, six autoantigens and two VV antigens were selected for differential serological analysis and titration: mouse homologues of known human autoantigens not represented in the SEREX database (MYH6 [29] and macrogolgin [GOLGB1; references 30 and 31]), mouse homologues of autoantigens present in the SEREX database (ROCK1, ROCK2, and hsj2/DNAJA1), the surface molecule procollagen XIII (COL13A1; reference 32), and the immunodominant VV antigens (39-kD antigen and A-type inclusion protein). The SEREX technique not only facilitates the profiling of autoimmune B cell responses, but also enables simple and rapid quantification of autoantibody titers using the recombinant protein in phage lysates. To this end, replica-plated phages were incubated with serial twofold dilutions of serum from virus-infected mice and processed according to the standard SEREX protocol. As shown in Fig. 2 A, infection of C57BL/6 mice with 2×10^6 PFU of the cytopathic VV elicited both strong antibody responses against the immunodominant VV 39-kD antigen and the Golgi-associated protein GOLGB1 with titers of $>1:16,000$ and $1:16,000$, respectively. VV-induced autoreactivity against MYH6 and ROCK2 was significantly lower (Fig. 2 A). It is worthy to note that preimmune sera from naive mice did not show specific reactivity against autoantigens or VV antigens (Fig. S1, available at <http://www.jem.org/cgi/content/full/jem.20040358/DC1>). LCMV high dose infection has been shown to induce strong polyclonal B cell activation with hypergammaglobulinemia and elevated autoantibody titers (33). Indeed, despite the rather high initial serum dilution of 1:500, we found that LCMV-induced IgG antibodies reacted weakly with the selected autoantigens and with VV antigens (Figs. 2 B and 3 A). However, both high dose (Fig. 2 B) and low dose (Fig. 3 A) LCMV infection also elicited high titer autoantibody responses against several distinct antigens such

as ROCK1 and ROCK2 (Figs. 2 B and 3 A). Low level LCMV-induced reactivity against VV antigens and self-antigens such as GOLGB1 was transient and peaked around day 20 after infection (Fig. 4 A), whereas anti-ROCK2 autoantibodies persisted for >100 d (Fig. 4 B). Similarly, VV-induced IgG antibodies against VV antigen and self-antigens persisted at rather high levels until 120 d after infection (Fig. 4). Infection with the cytopathic vesicular sto-

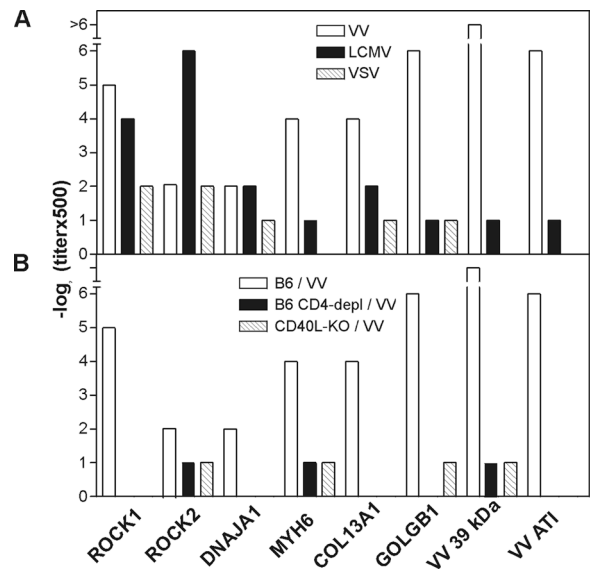


Figure 3. Antibody responses directed against a selected panel of autoantigens (ROCK1 and ROCK 2), hsj2 (DNAJA1), MYH6, COL13A1 and GOLGB1, and VV antigens (VV39kD, immunodominant antigen; VV ATI, A-type inclusion protein) as determined on day 21 after infection by quantitative phage assay. (A) Antibody titers in pooled sera from five C57BL/6 mice infected with 2×10^6 PFU VV, 200 PFU LCMV, and 2×10^6 PFU VSV. (B) The role of T helper cells in the induction of autoantibodies was determined by infection with 2×10^6 PFU VV of C57BL/6 mice, CD4-depleted mice, or CD40L-deficient mice.

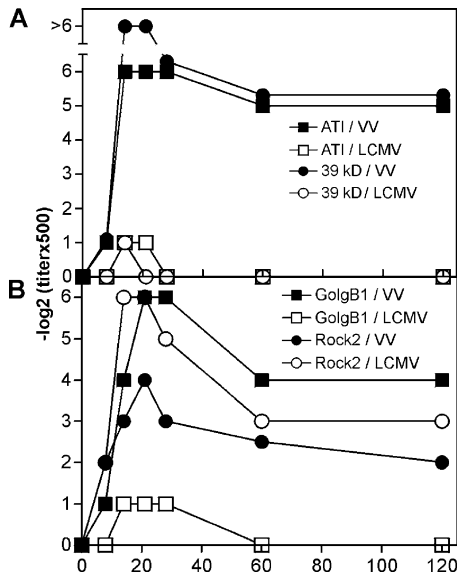


Figure 4. Time course of antibody responses against (A) VV proteins A-type inclusion protein and 39-kD immunodominant antigen, and (B) anti-self-antigens GOLGB1 and ROCK2 after infection of C57BL/6 mice with 2×10^6 PFU VV or 200 PFU LCMV. Pooled sera from five mice were assayed by quantitative phage assay at the indicated time points after infection.

matitis virus induced only weak autoantibody responses against the selected antigen panel and reactivity against VV antigens was not detectable (Fig. 3 A). Overall, these data indicate that virus infections elicit persisting “autoantibody fingerprints” in the infected host.

To investigate the role of T cell help for virus-induced autoantibody responses, CD4-depleted C57BL/6 mice (Fig. 2 B) were infected with VV and antibody titers against autoantigens and VV antigens were determined. Depletion of CD4 T cells nearly completely abolished the induction of both autoviral and antiviral antibody responses (Fig. 3 A). Similarly, VV-infected mice lacking the ligand for CD40, which is an essential molecule for Ig isotype switching, did not develop significant IgG responses against self- or viral antigens (Fig. 3 B), supporting the notion that virus-induced autoimmune B cell responses require specific T help delivered via CD40–CD40L interaction.

Discussion

The SEREX method has been shown to be a potent tool for the rapid detection and characterization of human tumor-associated antigens (34–36). To better understand how viral infections impact on the autoantibody profiles detected by SEREX analysis, we used sera from mice infected either with the cytopathic VV or the noncytopathic LCMV to screen libraries from different organs. Expression libraries were constructed from organ tissues of naive and virus-infected mice to contain not only the respective host cell transcriptomes, but also viral proteins and host-derived gene products induced upon infection of eukaryotic cells. Intriguingly, the majority of the cloned antibody specific-

ties are not restricted to our experimental mouse models, but are constituents of the human autoantibody repertoire.

The SEREX approach has been extensively applied by an international concerted action on a variety of different human cancer/serum sets. A multitude of tumor-associated autoantigens has been uncovered (37). However, it soon became apparent that suitable cancer-related antigens characterized by tumor-selective tissue distribution (38, 39) as well as by antibodies restricted to sera of tumor patients (40), comprise the minority of SEREX-identified antigens. Differential serology (41), i.e., comparatively probing SEREX antigens with sera from healthy controls and cancer patients, disclosed that a substantially larger number of antigens identified with SEREX are also recognized at comparable titers by sera of healthy individuals (42, 43). Thus, it is likely that most of the antibody reactivities mapped with crude sera of tumor patients occur independently from the neoplasm.

The data presented here clearly show that many of the human SEREX antigens have to be regarded as afterglows of infection-associated immunopathology, and that virus-induced autoimmunity may obscure truly tumor-specific antibody responses. The SEREX screening effort, though initiated with an entirely different aim, has thus en passant become the only systematic collection providing a nearly complete picture of comprehensive human adult IgG autoantibody repertoires shaped along life by encounters with triggers as diverse as viruses, tumors, and probably other causes of immunogenicity and tissue damage. This interpretation is supported by the recent work of T. Blankenstein’s group who found that immunization of mice with tumor cell lysate generates antibody responses against intracellular antigens comparable to those found in human patients (Blankenstein, T., personal communication).

Furthermore, our data support the notion that virus-mediated tissue damage leads to a massive anti-self-response that depends largely on the quality of the initial insult. Previously sequestered or immunologically ignored antigens are released and exposed to the immune system as a consequence of direct viral cytopathicity or through immune-mediated cell destruction. Therefore, the pattern of virus-induced de novo autoreactivity critically depends on virus-specific factors such as tissue and target cell tropism, as well as mechanisms of cell destruction. VV infection, for example, has been shown to favor the selection of hybridomas secreting autoantibodies against cytoskeletal proteins (44). The present analysis reveals that VV infection indeed precipitates strong B cell responses against this set of intracellular proteins, but also against a particular set of cell surface antigens, nuclear proteins, and heat shock proteins.

Production of a broad range of autoantibodies after viral infection may depend on polyclonal B cell activation such as bystander cytokines or through presentation of pinocytosed viral antigens to self-reactive B cells (45). However, our finding that the noncytopathic LCMV induced a distinct autoantibody pattern compared with the cytopathic VV infection argues against the interpretation that virus-induced autoimmune B cell responses are solely the result

of a general and nonspecific activation of the immune system. On the contrary, virus-specific patterns of autoreactivity on the B cell level indicate a role for antigen-driven selection in this process. Nearly all antigens were cloned multiple times independently. This not only mirrors abundance of the gene product in the screened tissue and high titer of the respective antibody but, most remarkably, can also be ascribed to the polyepitopic character of the B cell response against individual proteins, further supporting the notion that antigen-specific processes are operant.

We found autoantibody responses to be comparable to antiviral IgG responses in terms of their titer as well as their persistence. Most remarkably, intracellular proteins were clearly dominant, the only exceptions being COL13A1, hyaluronic acid binding protein, and secreted galectin 8 (LGALS8). Moreover, ubiquitous expression and wide tissue distribution rather than organ specificity is a shared feature of nearly all cloned autoantigens.

The majority of virus-induced autoantibodies are most likely not pathogenic, an interpretation that is supported by the clinical observation that autoantibody responses are frequent but autoimmune disease is rare. However, virus-induced autoantibodies may become pathogenic when cell surface structures (46, 47) or intracellular proteins deposited on the cell membrane are targeted (3, 4, 48). Furthermore, chronic or recurrent infections with selective virus-induced tissue destruction may lead to self-perpetuating cycles of tissue damage and exacerbation of autoimmunity (10). Therefore, detailed profiling of autoimmune B cell responses associated with viral infection might be important for the identification of etiologically significant autoantigens. Furthermore, the identification of virus-induced, specific autoantibody fingerprints and/or a negative list of "common" autoantigens may help to improve the predictive value of autoantibody tests (49).

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