Altered cell–cell and cell–matrix interactions in the development of systemic autoimmunity

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Abstract

MΦ of mice from the major inbred models of systemic lupus erythematosus (SLE) have an identical defect affecting the activity of the cytoskeletal regulator and G-protein Rho. This abnormality is triggered by apo cells. Strikingly, SLE-prone MΦ show normal Rho activity when cultured in the absence of apo cells. We used gene arrays to identify adhesion-related gene products that are abnormally expressed by MΦ from prediseased 4–6-week-old SLE-prone MRL mice in the presence of serum lipids mimicking apo cells (SL-Apo). MΦ of MRL mice differentially expressed 42 adhesion-related genes in the presence of SL-Apo. Of these, 32 were expressed normally in the absence of SL-Apo. As adhesive interactions play a major role in lymphocyte activation, the detected apo cell-dependent abnormality could predispose to the development of autoimmunity. Indeed, several recent genetic studies support a role for adhesion-related genes in the pathogenesis of chronic autoimmunity.

Keywords

Cytoskeleton; SLE; rodent; autoimmunity; gene array

Introduction

Apo cells comprise an abundant source of self-antigen (Ag). These Ag are the targets of autoantibodies (AAb) found in multiple autoimmune states, in particular systemic lupus erythematosus (SLE). While an intimate link between apo cell death and autoimmunity is widely accepted, the basis for this association remains unclear. Most attention has been

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directed toward derangements in apo cell clearance. Development of systemic autoimmunity in mice deficient in certain gene products involved in the clearance of apo targets supports this view. These studies have tended to focus on the persistence of apo corpses. However, the fact that impaired clearance also results in a loss or decrease of apo target-initiated signalling within the phagocyte must be taken into account. We have hypothesized that this second consequence of impaired apo cell clearance—namely, the diminution or absence of apo target-dependent proximal signalling events in responding phagocytes—plays a larger role in the development of autoimmunity [1].

We have previously shown that MΦ from mice of the major inbred models of SLE have an identical apo cell-dependent abnormality in the activity of the cytoplasmic G-protein Rho—a key regulator of the cytoskeleton—that results in profound abnormalities of adhesion and cytoskeletal organization [2–4]. Rho activity is severely diminished in MΦ from SLE-prone mice that have been incubated with apo targets. In contrast, Rho activity of the same MΦ is normalized and entirely comparable to that of non-autoimmune MΦ in the absence of apo targets. Thus, only in the presence of apo targets, whose uptake and processing leads to the presentation of self-Ag, SLE-prone MΦ evince an abnormality in Rho activity and in the cytoskeleton [2–4].

Here, we used gene arrays to explore the consequences of this apo target-dependent abnormality. We sought to identify gene products that were abnormally expressed by SLE-prone MRL solely in the presence of SL-Apo.

Materials and methods

PEC were derived from SLE-prone MRL/MpJ (MRL/+) or MRL/MpJ-*Tnfrsf6^{lpr}* (MRL/lpr) and from BALB/c mice (4–6 weeks; The Jackson Laboratory, Bar Harbor, ME, USA), as previously described [2–4]. Adherent cells, >98% MΦ, were cultured with 100 ng/ml lipopolysaccharide (LPS; *Escherichia coli* derived, serotype 0111:B4). Two variables were assessed: (i) culture in the presence or absence of serum lipids, and (ii) duration of LPS stimulation (0, 8, or 24 h). RNA was isolated using standard methods and was analysed with Affymetrix MG_U74Av2 mouse GeneChip® arrays and Micro-array Suite Version 5.0. Details may be obtained at <http://www.affymetrix.com>. Differentially expressed genes were identified using a strategy of recurrence and a threshold difference of 1.74-fold [5].

Results and discussion

MΦ from prediseased SLE-prone MRL mice differentially express multiple adhesion-related genes in the presence of SL-Apo

We used gene arrays to identify gene products that are abnormally expressed by SLE-derived MΦ in the presence of apo target cells and normally expressed in their absence. Given the apo cell-dependent abnormality in activity of the cytoplasmic G-protein Rho, as expressed by MΦ from SLE-prone mice [2–4], we focused our analysis on genes related to cellular adhesion and/or migration (Table I). We identified 42 genes whose differential expression by SLE-prone MRL vs. control BALB/c MΦ in the presence of SL-Apo exceeded a threshold of 1.75-fold in four independent comparisons at either 8 or 24 h

following LPS stimulation. The mean fold-difference in expression ($\pm$ standard error) for these 42 genes was 3.04 ± 0.27 .

We also determined the expression of these same 42 genes by MRL vs. BALB/c M Φ in the absence of SL-Apo. Comparison of their expression patterns under these two conditions (presence or absence of SL-Apo) allowed us to sort the 42 genes into two subsets with different pathophysiologic significance.

Genes in the first and larger subset ($n = 32$) were differentially expressed by MRL M Φ only when they were allowed to interact with SL-Apo. In the absence of SL-Apo, MRL M Φ expressed these 32 genes at a level comparable to that of BALB/c M Φ (<1.75 -fold). Differential expression for this subset of genes cannot be attributed to mutation or allelic variation between MRL and BALB/c mice, since their differential expression is conditional and correctable. Thus, in the absence of SL-Apo, expression of these 32 genes by MRL M Φ is normalized. Importantly, since genes in this subset are differentially expressed by MRL M Φ solely in the presence of SL-Apo, the consequences of their aberrant expression should be manifest only during those times when MRL M Φ are interacting with apo cells (or their surrogate, SL-Apo), an abundant source of self-Ag.

Genes in the second smaller subset ($n = 10$) were differentially expressed by MRL M Φ in both the presence and the absence of SL-Apo. Their differential expression is more likely to result from fixed strain-specific differences between MRL and BALB/c mice. Such differences may be functionally inconsequential. Alternatively, such differences may be indicative of a true SLE-associated allele or mutation, much like the *lpr* mutation of the Fas gene.

Abnormalities of adhesion can predispose to the development of systemic autoimmunity

The gene products in Table I may be sorted into groups according to function. The first and most provocative group is involved in cell–cell interactions. Examples include several cell surface adhesion receptors (e.g. VCAM-1, DM-GRASP, CD72, and the CD5-like CT-2) or bridging molecules like thrombospondin-1 that facilitate the interaction between M Φ and other cells.

Adhesive interactions play a major role in formation of the immunological synapse between T cell and antigen-presenting cell (APC; [6]). Alterations in the formation and strength of the immunological synapse modulate signalling events triggered by T-cell receptor engagement and help to determine the balance between tolerance and activation [7]. In this regard, it is noteworthy that MRL M Φ under-expressed CD72 by >10 -fold. CD72 is a cell surface glyco-protein primarily expressed by B cells that appears to modulate signalling through the B-cell receptor. One of its binding partners is CD5. Mice deficient in CD72 develop AAb to dsDNA and demonstrate abnormalities in the maintenance of peripheral tolerance by B cells [8]. Of note, CT-2, which possesses homology to CD5, was also differentially expressed by MRL M Φ .

The second group is involved in modulating cell–matrix adhesion and/or migration. Examples include constituents of the extracellular matrix (ECM), (e.g. decorin, $\alpha 1$ chain of

type I procollagen, and chondroitin sulfate proteoglycan 2) or secreted molecules that modify the ECM (e.g. urokinase, matrix metalloproteinases-9 and -13). Other examples include gene products that modulate the adhesiveness or directed migration of cells (e.g. MAGE-D1, metadherin, and TGF- β -induced 68 kDa protein). Abnormalities of genes in this group may affect the ability of M Φ to be recruited to sites of inflammation [9]. In addition, inhibition of adhesion through administration of a moAb to the adhesion receptor CD11b/CD18 abrogated autoimmunity in a model of type I diabetes [10].

The final group is involved in regulation of the actin cytoskeleton and microtubular network. Genes in this group have the capacity to modulate both cell–matrix and cell–cell interactions. Examples include several small cytoplasmic G-proteins (e.g. Rab5A, Rab7, and Rap2B) and their upstream GTPase activating proteins (Ras p21 protein activators 3 and 4, TBC1D15), as well as other upstream regulatory proteins (DOCK2, DOCK7, Kif23, centaurin- γ 2, and gelsolin-like actin filament capping protein). Other examples include several downstream targets of Rho (e.g. citron kinase) or Rho-family G-proteins (e.g. iNOS2A, Rsk3, subunit 5 of Arp2/3 complex, and stathmin 1).

Cytoskeletal rearrangements triggered by Rho-family G-proteins contribute to the co-stimulation and activation of lymphocytes [11]. Importantly, abnormalities of adhesion and Rho-family G-proteins have been described in M Φ and lymphocytes derived from patients and mice with SLE [3,4,9,12]. In strong support of a role for abnormalities of adhesion in the development of autoimmunity, two recent genetic studies on murine and human SLE have identified disease-modifying loci with strong links to adhesion. An inactivating mutation of coronin-1A, a multi-functional regulator of the cytoskeleton that participates in formation of the immunological synapse, conferred resistance to autoimmunity in mice [13]. In addition, polymorphisms within the locus for ITGAM (integrin alpha-M, or CD11b) conferred susceptibility to SLE in human SLE [14]. The ITGAM-containing CD11b/CD18 adhesion receptor is one of several M Φ receptors whose engagement in the presence of apo cells leads to impaired Rho activity and abnormalities of adhesion and cytoskeletal arrangement [3,4].

Conclusion

Adhesive interactions play a major role in immune homeostasis. We have previously reported that M Φ from prediseased SLE-prone mice have an identical abnormality that affects activity of the G-protein Rho. This abnormality is present only when M Φ interact with apo cells. Here, we extend these findings to show that MRL M Φ also differentially express multiple adhesion-related genes in the presence of SL-Apo. Of these, >75% showed normal expression in the absence of SL-Apo. As apo cells represent an abundant source of self-Ag, a M Φ abnormality triggered by interaction with apo cells (or their surrogate, SL-Apo) would be most prominent during the processing and presentation of self-Ag and could therefore predispose to autoimmunity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table I

Adhesion-related genes differentially expressed by MΦ of MRL vs. BALB/c mice.

Gene name	Gene function	Difference*
<i>Apoptotic cell-dependent differential gene expression</i>		
Over-expressed by MRL MΦ		
Microtubule-associated protein 6	Reg. of μ T NW	+3.7
MAGE-1	Inhibition of adhesion and motility	+3.0
Plexin	Reg. of axonal growth cone migration in CNS	+3.0
Kif5B	Reg. of μ T NW	+3.0
β -actin	Component of actin CS	+2.8
Kif23	Reg. of μ T NW; interacts with Cyk4, a RhoGAP	+2.8
Citron kinase	Reg. of actin CS; downstream effector of Rho	+2.6
Stathmin 1	Reg. of μ T NW; downstream effector of Rac/Cdc42	+2.3
C-type lectin, SF member 6	Lectin cell surface adhesion receptor	+2.3
DOCK2	Reg. of μ T NW; upstream regulator of Rac	+2.1
Rap2B	Ras family member; cell growth and differentiation	+2.1
Centaurin- γ 2	Reg. of the actin CS	+2.0
Arrestin β 1	Reg. of G protein-dependent signaling	+2.0
Ras p21 protein activator 3	RasGAP	+1.9
Lectin, galactose binding, sol. 1	Sol. receptor specific for β -galactose lectins	+1.7
Neuropilin	Reg. of axonal growth cone migration in CNS	+1.6
Under-expressed by MRL MΦ		
Chondroitin sulfate proteoglycan 2	ECM protein	-6.4
VCAM-1	Adhesion receptor	-4.9
HSP70	Expression is transcriptionally reg. by actin CS	-4.3
Metadherin	Adhesion receptor involved in adhesion/motility	-4.3
Decorin	ECM protein	-2.6
Matrix metalloproteinase 9	Modification of ECM	-2.6
Lectin, galactose binding, sol. 8	Sol. receptor specific for β -galactose lectins	-2.5
Plexin A3	Reg. of axonal growth cone migration in CNS	-2.3
Rab7	Ras SF member; vesicular transport	-2.1
Rab5A	Ras SF member; vesicular transport	-2.1
Procollagen, type I, α 1	ECM protein	-2.1
DOCK7	Reg. of μ T NW; upstream regulator of Rac	-2.0
Gelsolin-like actin filament capping protein	Reg. of actin CS	-2.0
Rsk3	Downstream effector of Cdc42	-2.0
Arp 2/3 complex, subunit 5	Reg. of actin CS; downstream effector of Rac/Cdc42	-2.0
Ras p21 protein activator 4	RasGAP	-1.9
<i>Fixed differential expression[†]</i>		
Over-expressed by MRL MΦ		
CT-2	Adhesion receptor mediating cell-cell interactions	+6.1

Gene name	Gene function	Difference [*]
Matrix metalloproteinase 13	Modification of ECM	+5.7
TGF- β -induced, 68 kDa	Inhibitor of adhesion induced by TGF- β	+4.3
TBC1D15	RabGAP	+2.5
DM-GRASP	Cell surface adhesion receptor	+2.0
Under-expressed by MRL M Φ		
CD72	Adhesion receptor mediating cell–cell interactions	–11.3
iNOS2A	Downstream effector of Rac	–3.5
Thrombospondin 1	Secreted molecule mediating cell–cell interactions	–2.6
Urokinase	Modification of ECM	–2.5
Integrin- β 1 binding protein 1	Reg. of integrin-mediated adhesion	–2.3

CS = cytoskeleton

μ _T NW = microtubular network

Reg. = regulator

Sol. = soluble

^{*} Differences were determined at 8 and 24 h after stimulation, and the greater of these values is given. Over- and under-expression by MRL M Φ are indicated by plus and minus signs, respectively;

[†] Differences were determined in presence and absence of SL-Apo. Differences for genes listed in “Fixed differential expression” exceeded 1.75 for both conditions. Differences for genes in “Apoptotic cell-dependent differential expression” exceeded 1.75 only in presence of SL-Apo.