Markey pathway-
Viruses and arthritis

Debbie Lenschow M.D., Ph.D.
August 30, 2012
Autoimmune disease:

Breakdown of tolerance to self proteins such that the immune system attacks self tissue causing disease.
General concepts of Autoimmunity

• Autoimmunity results from a failure or breakdown of the mechanisms normally responsible for maintaining self-tolerance in B cells, T cells, or both.

• The major factors that contribute to the development of autoimmunity are genetic susceptibility and environmental triggers, such as infection.

• Autoimmune disease can be either systemic or tissue specific.

• Various effector mechanisms are responsible for tissue injury in different autoimmune diseases.
Why do we think Rheumatoid Arthritis is an autoimmune disease?

- Autoantibodies present in the majority of patients:
  - Rheumatoid factor- autoAbs directed against Fc fragment of IgG
  - anti-CCP Abs- cyclic citrullinated peptide
- Systemic disease affecting multiple joints and other tissues.
- Activated lymphocytes, monocytes, and macrophages are present in the inflammed joints.
- Marked association between RA and certain HLA subtypes
- More common in women than men.
- Similarities to murine model of collagen-induced arthritis.
Etiology of RA/Autoimmunity
Genotype
(for example, AIRE mutation)
Table 1 | Simple genetic traits associated with autoimmunity

<table>
<thead>
<tr>
<th>Gene</th>
<th>Human disease</th>
<th>Mouse mutant or knockout</th>
<th>Mechanism of autoimmunity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIRE</td>
<td>APS-I (Autoimmune polyendocrine syndrome)</td>
<td>Knockout</td>
<td>Decreased expression of self-antigens in the thymus, resulting in defective negative selection of self-reactive T cells</td>
<td>2, 3</td>
</tr>
<tr>
<td>CTLA4</td>
<td>Association with Graves’ disease, type 1 diabetes and others</td>
<td>Knockout</td>
<td>Failure of T cell anergy and reduced activation threshold of self-reactive T cells</td>
<td>9, 65, 66</td>
</tr>
<tr>
<td>FOXP3</td>
<td>IPEX (scurfy) (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome)</td>
<td>Knockout and mutation</td>
<td>Decreased generation of CD4&lt;sup&gt;+&lt;/sup&gt; CD25&lt;sup&gt;+&lt;/sup&gt; regulatory T cells</td>
<td>11-13, 67</td>
</tr>
<tr>
<td>FAS, FASL</td>
<td>ALPS Autoimmune lymphoproliferative syndrome</td>
<td>lpr/lpr, glc/glc mutants</td>
<td>Failure of apoptotic death of self-reactive B and T cells</td>
<td>16, 68</td>
</tr>
<tr>
<td>C4 complement protein</td>
<td>Associated with SLE</td>
<td>Knockout</td>
<td>Defective clearance of immune complexes and possible failure of B cell tolerance</td>
<td>Reviewed in ref. 69</td>
</tr>
</tbody>
</table>

Genetic and epigenetic components determine gene function in health and disease

Ballestar, E. (2011) Epigenetic alterations in autoimmune rheumatic diseases
Nat. Rev. Rheumatol. doi:10.1038/nrrheum.2011.16
The strongest associated gene with autoimmune diseases is MHC

<table>
<thead>
<tr>
<th>Disease</th>
<th>HLA allele</th>
<th>Relative Risk*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>DR4</td>
<td>4.2</td>
</tr>
<tr>
<td>IDDM</td>
<td>DR3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>DR4</td>
<td>5-6</td>
</tr>
<tr>
<td></td>
<td>DR3/DR4 het</td>
<td>25</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>DR2</td>
<td>4.8</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>DR2/DR3</td>
<td>5.8</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
<td>DR4</td>
<td>14.4</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>B27</td>
<td>87.4</td>
</tr>
</tbody>
</table>

* Relative risk is defined as the probability of development of a disease in individuals with a particular HLA allele versus individuals lacking that HLA allele.
How strong is the genetic component in RA?

- Prevalence of RA in the general population: 0.5-1.0%
- Monozygotic twin concordance rates in RA: 12-15%
- Prevalence of disease in siblings of affected individuals: 2-3%
- Relative risk to siblings of affected individuals: 3-12%
  - if seropositive, erosive RA: 5-10%
How strong is the genetic component in RA?

- Prevalence of RA in the general population: 0.5-1.0%
- Monozygotic twin concordance rates in RA: 12-15%
- Prevalence of disease in siblings of affected individuals: 2-3%
- Relative risk to siblings of affected individuals: 3-12%
  if seropositive, erosive RA: 5-10%

Low level of concordance indicates additional factors, such as environment, are involved.
Genetic and epigenetic components determine gene function in health and disease

Ballestar, E. (2011) Epigenetic alterations in autoimmune rheumatic diseases 
Hormonal influences

- Many autoimmune disease have a higher incidence in females than in males.
- Often the course of disease can be impacted upon by pregnancy.
- Mechanism is unknown
Chemicals

- Anatomic alterations in tissues due to trauma or ischemic injury can lead to exposure of self antigens that are normally sequestered from the immune response.
- Inflammation or exposure to chemical can lead to structural alterations in self proteins and the formation of new determinants that the immune system responds against.
• Chromatin from dying/apoptotic cells exhibit modifications
  – Unique histone phosphorylation
  – Aberrant DNA methylation
  – Oxidative DNA damage

• Protein autoantigens undergo posttranslational modifications
  – Citrullination
  – Oxidative fragmentation

→ Generation of novel autoantigen epitopes
Genetic and epigenetic components determine gene function in health and disease

Ballestar, E. (2011) Epigenetic alterations in autoimmune rheumatic diseases
Nat. Rev. Rheumatol. doi:10.1038/nrrheum.2011.16
Difficulty in establishing a direct epidemiological association between microbial infections and autoimmunity

- Most infections are cleared by the time of disease diagnosis ("hit and run" events)
- Infections may not initiate autoimmunity, but instead may accelerate a pre-existing autoimmune condition to progress to clinical disease.
- Genetic factors, such as MHC haplotype can profoundly influence the antiviral immune response
- Precise timing of infection, magnitude of inflammation, viral strain may all have an important role.
Genetic and epigenetic components determine gene function in health and disease

Ballestar, E. (2011) Epigenetic alterations in autoimmune rheumatic diseases

- Molecular mimicry
- Adjuvant affect
- Bystander activation
- Chronic infection (alphaviruses)
Link between infection and autoimmunity

1) Molecular mimicry (antigen specific)
2) Adjuvant effect (antigen nonspecific)
   - activation of APCs by microbial products
3) Bystander activation (antigen nonspecific)
   - abnormal release of antigen due to microbial induced damage
4) Autoimmune diseases are chronic infections
Molecular Mimicry - Term used to describe what happens when a T- or B-cell receptor recognizes a microbial peptide that is structurally similar to a self peptide. The immune response is initially directed at the microbial peptide and then spreads to tissues that present cross-reactive self peptide, resulting in autoimmunity.
Molecular mimicry

Roughly 5% of mAbs made to 15 different viruses cross-reacted with “host-self determinant”

<table>
<thead>
<tr>
<th>Virus</th>
<th>No. Tested</th>
<th>Reactive with Uninfected Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coxsackie B</td>
<td>68</td>
<td>3</td>
</tr>
<tr>
<td>Japanese Encephalitis</td>
<td>34</td>
<td>6</td>
</tr>
<tr>
<td>Lymphocytic</td>
<td>174</td>
<td>3</td>
</tr>
<tr>
<td>Choriomeningitis</td>
<td>64</td>
<td>9</td>
</tr>
<tr>
<td>Theiler’s Virus</td>
<td>39</td>
<td>5</td>
</tr>
<tr>
<td>Measles</td>
<td>80</td>
<td>2</td>
</tr>
<tr>
<td>Rabies</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>Vesicular Stomatitis</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Herpes Simplex (l)</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Vaccinia</td>
<td>134</td>
<td>2</td>
</tr>
<tr>
<td>Dengue</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Human Immunodeficiency Virus</td>
<td>110</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>815</td>
<td>45</td>
</tr>
</tbody>
</table>
Molecular mimicry in humans has been reported in two diseases:

- Rheumatic fever
- Guillain-Barre syndrome
Rheumatic heart disease

- Following infection with group A β-hemolytic streptococci which causes streptococcal pharyngitis, untreated individual can develop rheumatic fever characterized by inflammation affecting all layers of the heart.
- Sera of patients with acute rheumatic fever contained heart reactive autoantibodies.
- Myocardial tissue of rheumatic heart disease patients contained bound Ig reactive with streptococci.
- Cross-reactive epitope derived from type 5 streptococcal M protein reacted with cardiac myosin and tropomyosin.
Rheumatic heart disease

• More recent studies from Kalil’s group has demonstrated that T cell derived from inflammatory foci in the heart recognize both cardiac proteins and epitopes from streptococcal M protein.
Molecular mimicry in RA

- Microorganisms implicated in RA include *Proteus mirabilis, Mycobacterium tuberculosis,* and Epstein Barr virus (EBV).
- Antibodies specific for Epstein-Barr nuclear antigen-1 (EBNA-1) have been shown to cross-react with a 62 kDa synovial membrane protein.
- Neither epidemiologic nor serologic investigations have provided really substantial support for molecular mimicry in RA disease pathogenesis.
Link between infection and autoimmunity

1) Molecular mimicry (antigen specific)

2) Adjuvant effect (antigen nonspecific)
   - activation of APCs by microbial products

3) Bystander activation (antigen nonspecific)
   - abnormal release of antigen due to microbial induced damage

4) Autoimmune diseases are chronic infections
Recognition by the innate immune system

- Target of innate immune recognition are conserved molecular pattern of microorganisms (PAMPs)
- PAMPS are generated by the organism, not the host
- PAMPs are essential for microbial survival and are therefore conserved structures allowing for recognition with limited number of receptors
- Pattern recognition receptors include toll-like receptors (TLRs), NACHT-LRR families (NLRs), and RNA helicases (RLRs).
TLRs

TNFα, IL-6, IL-1
Role of proinflammatory cytokines in autoimmune disease

• Several of the therapies currently utilized in the treatment of RA target proinflammatory cytokines- TNFα, IL-6, IL-1

• Type I IFNs are elevated in patients with active SLE and dermatomyositis, and studies in mice have demonstrated a potential role for IFNs in the pathogenesis of SLE.
### Table 1 Genetic elements that are hypomethylated in autoimmune rheumatic diseases

<table>
<thead>
<tr>
<th>Genetic element</th>
<th>Disease</th>
<th>Cell type</th>
<th>Product and/or function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITGAL</td>
<td>SLE</td>
<td>CD4⁺ T cell</td>
<td>Integrin α-L, important for cell–cell adhesion</td>
<td>Lu et al. (2002)&lt;sup&gt;67&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD70</td>
<td>SLE</td>
<td>CD4⁺ T cell</td>
<td>CD70 antigen, required for T cell proliferation</td>
<td>Oelke et al. (2004)&lt;sup&gt;68&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD40LG</td>
<td>SLE</td>
<td>CD4⁺ T cell</td>
<td>CD40 ligand, stimulates overproduction of IgG by B cells</td>
<td>Lu et al. (2007)&lt;sup&gt;69&lt;/sup&gt;</td>
</tr>
<tr>
<td>PRF1</td>
<td>SLE</td>
<td>CD4⁺ T cell</td>
<td>Perforin 1, involved in autoreactive killing</td>
<td>Kaplan et al. (2004)&lt;sup&gt;70&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD5</td>
<td>SLE</td>
<td>CD19⁺ B cell</td>
<td>T-cell surface glycoprotein CD5, associated with production of several interleukins, negative regulator of BCR signaling</td>
<td>Garaud et al. (2009)&lt;sup&gt;71&lt;/sup&gt;</td>
</tr>
<tr>
<td>IFNGR2</td>
<td>SLE</td>
<td>PBMC</td>
<td>IFN-γ receptor 1, proinflammatory activity through interaction with different cell types</td>
<td>Javiere et al. (2010)&lt;sup&gt;76&lt;/sup&gt;</td>
</tr>
<tr>
<td>MMP14</td>
<td>SLE</td>
<td>PBMC</td>
<td>MMP-14, involved in tissue destruction and inflammation</td>
<td>Javiere et al. (2010)&lt;sup&gt;76&lt;/sup&gt;</td>
</tr>
<tr>
<td>LCN2</td>
<td>SLE</td>
<td>PBMC</td>
<td>Neutrophil gelatinase-associated lipocalin, iron transporter and marker for SLE</td>
<td>Javiere et al. (2010)&lt;sup&gt;76&lt;/sup&gt;</td>
</tr>
<tr>
<td>KIR3DL1</td>
<td>SLE</td>
<td>SLE</td>
<td>Killer cell Ig-like receptor, three domains, long cytoplasmic tail, 1, modulates NK cell-mediated killing</td>
<td>Basu et al. (2009)&lt;sup&gt;88&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>miRNAs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miRNA203</td>
<td>RA</td>
<td>PBMC</td>
<td>IL-6, participates in B cell response</td>
<td>Nile et al. (2009)&lt;sup&gt;72&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Repetitive elements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rDNA (18S, 28S)</td>
<td>SLE</td>
<td>PBMC</td>
<td>Constitutive elements of ribosomal particles</td>
<td>Javiere et al. (2010)&lt;sup&gt;76&lt;/sup&gt;</td>
</tr>
<tr>
<td>L1 elements</td>
<td>RA</td>
<td>SF</td>
<td>Fine tuning of expression for nearby or enveloping genes</td>
<td>Karouzas et al. (2009)&lt;sup&gt;74&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: BCR, B cell receptor; IFN, interferon; Ig, immunoglobulin; IL, interleukin; miRNA, microRNA; MMP, matrix metalloproteinase; NK, natural killer; RA, rheumatoid arthritis; rDNA, ribosomal DNA; SF, synovial fibroblast; SLE, systemic lupus erythematosus; PBMC, peripheral blood mononuclear cell.
Viruses induced activation of the innate immune system. In a genetically susceptible person this response may be overactive and then result in immunopathology or autoimmunity.
How are these receptors related to autoimmunity

- The NLR- NOD2, missense mutations are associated with two autoimmune disease, Crohn’s disease and Blau syndrome.

- The NLR- NALP3/cryopyrin/CIAS1 associated with 3 autoimmune diseases, familial-cold autoinflammatory syndrome, Muckle-Wells, syndrome, and neonatal onset multisystem inflammatory disease.

- Mutations in pyrin gene is mutated in patients with Mediterranean fever.

- MDA5 (RLR) mutations have been linked to type I DM
Link between infection and autoimmunity

1) Molecular mimicry (antigen specific)
2) Adjuvant effect (antigen nonspecific)
   - activation of APCs by microbial products
3) Bystander activation (antigen nonspecific)
   - abnormal release of antigen due to microbial induced damage
4) Autoimmune diseases are chronic infections
Bystander activation of autoreactive cells

Normally an APC presenting self antigen will not activate T cells because they are not activated or therefore don’t express costimulatory molecules.

Self antigens are released and the APCs can be activated to present to autoreactive T cells secondary to the damage induced by the infection.
Link between infection and autoimmunity

1) Molecular mimicry (antigen specific)
2) Bystander activation (antigen nonspecific)
   - abnormal release of antigen due to microbial induced damage
3) Adjuvant effect (antigen nonspecific)
   - activation of APCs by microbial products
4) Autoimmune diseases are chronic infections
Alphaviruses

• Arboviruses (transmitted by mosquito) belonging to the Togaviridae family
• Enveloped, linear, positive-sense, single-stranded RNA viruses
• Alphavirus group contains 28 viruses, six of which cause human joint disorders

  – Chikungunya virus (South and East Asia, Africa, West Pacific)*
  – O’ngyong-nyong virus (central Africa)
  – Ross River and Barmah Forest (Australia, Pacific)
  – Sindbis virus (Africa, Asia, Australia)
  – Mayaro virus (South America, French Guyana)
Alphaviruses associated with rheumatic disease

Table 1 | Alphaviruses associated with rheumatic disease

<table>
<thead>
<tr>
<th>Virus</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chikungunya virus</td>
<td>Large sporadic epidemics</td>
</tr>
<tr>
<td>Ross River virus</td>
<td>Mean of ≈4,000 cases per annum in Australia&lt;sup&gt;17&lt;/sup&gt; An epidemic occurred in 1979–1980 &gt;60,000 cases in some of the Pacific Islands&lt;sup&gt;18&lt;/sup&gt;</td>
</tr>
<tr>
<td>Barmah Forest virus</td>
<td>Mean of ≈1,000 cases per annum in Australia&lt;sup&gt;18&lt;/sup&gt;</td>
</tr>
<tr>
<td>O’nyong-nyong virus</td>
<td>Rare epidemics, &gt;2 million cases in 1959–1961&lt;sup&gt;30,104&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mayaro virus</td>
<td>Occasional small outbreaks (30–100 cases)&lt;sup&gt;24,122&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Sindbis virus</em>&lt;sup&gt;2,21,22&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Karelian fever</td>
<td>Rare (Karelia, West Russia)</td>
</tr>
<tr>
<td>Ockelbo virus</td>
<td>Mean ≈30 cases per annum (Sweden)</td>
</tr>
<tr>
<td>Pogosta virus</td>
<td>Mean ≈140 (range 1–1,282) cases per annum (Finland)</td>
</tr>
</tbody>
</table>

For geographical distribution of these disease outbreaks see Figure 1.

*Nat. Rev. Rheumatol.* doi:10.1038/nrrheum.2012.64
Approximate geographical locations of diseases associated with arthritogenic alphaviruses

Suhrbier, A. et al. (2012) Arthritogenic alphaviruses—an overview
Nat. Rev. Rheumatol. doi:10.1038/nrrheum.2012.64
Chikungunya virus

• From the African dialect Swahili or Madonde means “to walk bent over”, referring to the incapacitating arthralgias experienced by these patients. In Congo, the disease is called buka-buka meaning “broken-broken”.

• First isolated in 1953 in Tanzania during an outbreak of febrile polyarthritis.

• Tropical disease, unknown by most people in the world, including medical doctors, until 2004.

• Before 2000, only a few benign, imported infections had been seen in North America and Europe.
Clinical syndrome- CHIKV fever

• Short incubation (2-6 days) until symptom onset
• During acute infection there are high levels of viremia
  \((10^8-10^{12} \text{ virus per ml of blood})\)

• Acute stage-
  – sudden onset of high fever (100%),
  – incapacitating polyarthritis (100%) (bilateral, symmetric, cumulative within a few days, involving the peripheral joints: hands, wrists, feet, ankles),
  – maculopapular rash (50%) with diffuse hyperemia and edema of the face and extremities.
  – In children rash presents as a bullous rash with pronounced sloughing and localized petechiae.
Usual course of CHIKV disease in adults

Suhrbier, A. et al. (2012) Arthritogenic alphaviruses—an overview
Nat. Rev. Rheumatol. doi:10.1038/nrrheum.2012.64
Some patients develop persisting, erratic, relapsing polyarthalgias, resulting in severe incapacitation for weeks to more than 1 year.

- Radiographic findings are usually normal (no erosions)

- Biological markers of inflammation (ESR or CRP) are normal or mildly elevated
Clinical testing for CHIKV

- In the acute phase viremia can be detected in blood samples in EDTA tube by either culture or RT-PCR (days 0-7). In some patients during the acute phase viral titers in the serum reach $10^9$ pfu/ml. Sensitivity in culturing the virus 5 days after disease onset decreases. RT-PCR is faster, more sensitive than culture and is specific.

- Anti-CHIKV IgM are detected by ELISA from the fifth day after the onset of symptoms. These persists for several weeks to 3 months.

- Anti-CHIKV IgG are detected within a few weeks by ELISA. IgG is detected in convalescent samples and can persists for years.

- The sensitivity and specificity of these tests are poorly established. Cross-reactivity with antigens from O’nyong-nyong, Mayaro, and Ross River virus may lead to false positives.
Treatment/Prevention of CHIKV

- No effective antiviral treatment
- Symptomatic treatment with NSAIDs for arthritis
- Trial in southern Africa failed to confirm efficacy of chloroquine on arthralgias.

- Large scale prevention campaigns using DDT have been effective against *A aegypti* but not *A albopictus*.
- No commercial vaccine available. Vaccines are now under development.
Recent Chikungunya virus Outbreak

• In 2004, ongoing CHIKV outbreak began in Lamu Island, Kenya (13,500 people infected- attack rate higher than 50%).
• Spread to Comoros Islands, Reunion, Mayotte, Maritius, and Madagascar
• By December 2007, several million cases reported in India, with continued expansion into Sri Lanka, Indonesia, and Malaysia
• By 2007, the virus reached Europe.
• Since 2004 more than 1000 travelers have been diagnosed with CHIK fever, with most patients consulting their doctors in the chronic stage.
Reason for efficiency of CHIKV extension

- Adaptive mutation at position 226 of E1 gene from alanine to valine
  - Improved adaptation of CHIKV to *A. albopictus*, including into the salivary glands
  - Improved transmission to vertebrate species
Reunion Island outbreak

September 2006- 266,000 residents (population of 770,000) were infected.

Acute Disease
• Excess deaths were reported during outbreak with estimated case-fatality rate for CHIK disease 0.3 to 1/1000 (mainly in people over 75 yrs of age).

• Description of 123 severe cases. Direct role of CHIKV limited to acute CNS (meningoencephalitis) or cardiac complications.

• Increased risk of abortion in the first trimester.

• Mother to child transmission seen with 50% of mothers with ongoing CHIKV infection at the time of delivery. 27 of 35 documented cases of neonatal CHIKV were severe.

• Infected neonates had long term sequelae.
Reunion Island outbreak

Chronic Disease:

Five year longitudinal study from La Réunion:
• Chronic symptoms seen in 57% of infected patients at 15 months; 12% after 3-5 years

• Manifested as joint pain, stiffness and swelling
  – (Ankles > Wrists > Knees > Fingers > Feet)

• Risk Factors:
  – Age > 45
  – Osteoarthritis
  – Severe initial pain

It can mimic RA. In a study from La Reunion, 22 cases met ACR criteria for RA.
How do alphaviruses induced arthritis?

Are there differences in the pathogenesis of acute vs. chronic disease?

Does viral infection induce the development of autoimmunity?
Acute infection

• Most alphaviruses share tropism for joints and induce similar polyarthralgia/arthritis
CHIKV Immunolabeling in Human Tissue Samples during acute infection

Virus replicates in connective tissue fibroblasts and myoblasts
Human infants infected with CHIKV mount a strong acute proinflammatory cytokine/chemokine response.
Murine models of alphavirus infection

**Neonatal**
- Severe, disseminated infection
- High viral titers in multiple organs
- Lethality in mice up to 9-10 days of age

- Infection of 2-4 week old mice results in joint inflammation and myositis.
- No lethality

**Adolescent/Adult**
- Infection of 2-4 week old mice results in joint inflammation and myositis.
- No lethality
RRV model of arthritis/myositis

A

B

Characterization of Ross River Virus Tropism and Virus-Induced Inflammation in a Mouse Model of Viral Arthritis and Myositis

Thomas E. Morrison,1,2,3 Alan C. Whitmore,3 Reed S. Shabman,1,2,3 Brett A. Lidbury,4 Suresh Mahalingam,4 and Mark T. Heise1.
Host pathways involved in the regulation of alphavirus induced arthritis/myositis

Infiltration into the muscle of monocytes, macrophages, T cells, and NK cells

Similar disease developed in RAG-/- mice indicating T and B cells were not required for disease

Proinflammatory cytokines (IL-6, TNF) were produced

Complement pathway was involved in disease pathogenesis
Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)
Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)
RRV-induced disease is less severe in C3−/− mice.

WT and C3−/− mice had similar viral loads in the muscle and joints at all time points
Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)
Figure 1. MBL is required for development of severe RRV-induced disease and tissue damage.

http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1002586
Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)
Figure 1. MBL is required for development of severe RRV-induced disease and tissue damage.
Figure 2. RRV infection induces MBL deposition onto cells, resulting in MBL-dependent C3 deposition onto infected tissues.

http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1002586
Figure 2. RRV infection induces MBL deposition onto cells, resulting in MBL-dependent C3 deposition onto infected tissues.

http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1002586
Figure 3. MBL deficiency does not affect viral replication or tropism within infected tissues.


http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1002586
Figure 4. MBL deficiency does not affect inflammatory cell recruitment, but alters expression of inflammatory mediators within the RRV infected muscle.

http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1002586
Figure 4. MBL deficiency does not affect inflammatory cell recruitment, but alters expression of inflammatory mediators within the RRV infected muscle.

http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1002586
Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)
What about the other host factors identified?
What about the other host factors identified?

Depletion of macrophages protected mice from RRV induced disease development.

Inhibition of chemokine synthesis protected mice from RRV myositis.

Are these findings relevant to human disease?
C3a levels are higher in synovial fluid from humans suffering from RRV-induced polyarthritis than in synovial fluid from humans with noninflammatory osteoarthritis.

Morrison T E et al. J. Virol. 2007;81:5132-5143
Figure 5. Levels of MBL are elevated in RRV patients.

No changes in the levels of C1q-C4 complexes or Bb in the serum

http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1002586
Figure 5. Levels of MBL are elevated in RRV patients.
Chronic disease

Pathophysiology and chronic rheumatism following alphaviral infection remains unclear.

Is chronic disease due to persistent viral infection?

Does alphavirus infection induce other autoimmune diseases such as RA?
Patients with chronic disease following CHIKV infection display increased proinflammatory cytokines/chemokines.
Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages

Table 1
Virological and clinical outcomes were dependent on inoculation dose

<table>
<thead>
<tr>
<th>n</th>
<th>Dose (PFU)</th>
<th>Peak viremia</th>
<th>Clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day</td>
<td>vRNA copies/ml</td>
</tr>
<tr>
<td>3</td>
<td>$10^8$</td>
<td>1</td>
<td>$&gt;10^{10}$</td>
</tr>
<tr>
<td>1</td>
<td>$10^8$</td>
<td>&lt;2</td>
<td>$5 \times 10^8$</td>
</tr>
<tr>
<td>1</td>
<td>$10^7$</td>
<td>1</td>
<td>$5 \times 10^9$</td>
</tr>
<tr>
<td>1</td>
<td>$10^6$</td>
<td>2</td>
<td>$10^9$</td>
</tr>
<tr>
<td>1</td>
<td>$10^5$</td>
<td>2</td>
<td>$5 \times 10^8$</td>
</tr>
<tr>
<td>3</td>
<td>$10^3$</td>
<td>2–3</td>
<td>$5 \times 10^8$</td>
</tr>
<tr>
<td>1</td>
<td>$10^2$</td>
<td>3</td>
<td>$10^8$</td>
</tr>
<tr>
<td>1</td>
<td>$10^1$</td>
<td>4</td>
<td>$10^8$</td>
</tr>
<tr>
<td>1</td>
<td>$10^1$</td>
<td>No</td>
<td>Negative</td>
</tr>
</tbody>
</table>

High dose

Moderate dose

Low dose

Figure 4. Kinetics of plasma inflammatory mediators

Late in infection, multifocal necrosis seen with infiltration of monocytes and macrophages

CHIKV antigen and replicating virus can be detected late during the course of infection in macrophages, DCs, endothelial cells.
Follow up studies of the current epidemic in La Reunion and India will provide further insight into the epidemiology and possibly the pathogenesis of chronic arthritis induced by CHIKV.

Will patient infected with CHIKV have a higher incidence of autoantibody production or the development of RA?

Do patients with chronic CHIKV arthritis have persistent viral replication that triggers their disease?

Will treatment with complement inhibitors, chemokine inhibitors, anti-cytokine agents have an impact on acute vs. chronic disease induced by CHIKV?