Gene profiling of chikungunya virus arthritis in a mouse model reveals significant overlap with rheumatoid arthritis

Helder Nakaya, Emory University
Joy Gardner, University of Queensland
Yee-Suan Poo, University of Queensland
Lee Major, University of Queensland
Bali Pulendran, Emory University
Andreas Suhrbier, University of Queensland

Journal Title: Arthritis and Rheumatism
Volume: Volume 64, Number 11
Publisher: Wiley | 2012-11-01, Pages 3553-3563
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1002/art.34631
Permanent URL: https://pid.emory.edu/ark:/25593/v12fx

Final published version: http://dx.doi.org/10.1002/art.34631

Copyright information:
Copyright © 2012 by the American College of Rheumatology.

Accessed February 13, 2020 5:49 AM EST
Gene Profiling of Chikungunya Virus Arthritis in a Mouse Model Reveals Significant Overlap With Rheumatoid Arthritis

Helder I. Nakaya¹, Joy Gardner², Yee-Suan Poo², Lee Major², Bali Pulendran¹, and Andreas Suhrbier²
¹Emory Vaccine Center at Yerkes National Primate Research Center and Emory University, Atlanta, Georgia
²Queensland Institute of Medical Research and Australian Centre for Vaccine Development, Brisbane, Queensland, Australia

Abstract

Objective—Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes a chronic debilitating polyarthritis, for which current treatments are often inadequate. To assess whether new drugs being developed for rheumatoid arthritis (RA) might find utility in the treatment of alphaviral arthritides, we sought to determine whether the inflammatory gene expression signature of CHIKV arthritis shows any similarities with RA or collagen-induced arthritis (CIA), a mouse model of RA.

Methods—Using a recently developed animal model of CHIKV arthritis in adult wild-type mice, we generated a consensus CHIKV arthritis gene expression signature, which was used to interrogate publicly available microarray studies of RA and CIA. Pathway analyses were then performed using the overlapping gene signatures.

Results—Gene set enrichment analysis showed that there was a highly significant overlap in the differentially expressed genes in the CHIKV arthritis model and in RA. This concordance also increased with the severity of RA, as measured by the inflammation score. A highly significant overlap was also seen between CHIKV arthritis and CIA. Pathway analysis revealed that the overlap between these arthritides was spread over a range of different inflammatory processes. Involvement of T cells and interferon-γ (IFNγ) in CHIKV arthritis was confirmed in studies of MHCII-deficient mice and IFNγ-deficient mice, respectively.

Conclusion—These results suggest that RA, a chronic autoimmune arthritis, and CHIKV disease, usually a self-limiting viral arthropathy, share multiple inflammatory processes. New drugs and biologic therapies being developed for RA may thus find application in the treatment of alphaviral arthritides.

Chikungunya virus (CHIKV) is a mosquito-borne, single-stranded, positive-sense RNA virus (genus alphavirus) that has caused sporadic outbreaks of predominantly rheumatic
disease every 2–50 years, primarily in Africa and Asia (1). Most recently, during 2004–2011, CHIKV produced the largest epidemic ever recorded for an alphavirus, affecting an estimated 1.4–6 million patients. Imported cases were also reported in nearly 40 countries, including Europe, Japan, and the US.

The word “chikungunya” is derived from the Makonde language (Tanzania) and means “that which bends up,” referring to the severe joint pain–induced posture of afflicted individuals (1,2). CHIKV belongs to a group of mosquito-borne arthritogenic alphaviruses, which include the Australasian Ross River virus (RRV) and Barmah Forest virus, the African o’nyong-nyong virus, and the European Ockelbo and Pogosta viruses (1). Although many viruses can cause arthralgia/arthritis, none do so with the reliability of these alphaviruses. In adults, symptomatic infections are nearly always associated with arthropathy (1,3). The disease is characterized by acute and chronic polyarthritis/polyarthralgia, which is usually symmetric and often incapacitating. Other symptoms, such as fever, rash, myalgia, and/or fatigue, are often also present during the acute phase (1). The joints most commonly affected are multiple peripheral small joints (interphalangeal joints, wrists, and ankles) and large joints such as the shoulders, knees, and spine. The arthropathy usually resolves progressively over several weeks to months, usually without long-term sequelae, although CHIKV can sometimes produce severe disease manifestations and mortality (1,2). Chronic alpha-viral rheumatic disease is likely due to the persistence of viral replication in the joint tissue (1,4–6), with no evidence that autoimmune responses are responsible (3).

Alphaviral arthritides are generally treated with simple analgesics, such as paracetamol and/or nonsteroidal antiinflammatory drugs (NSAIDs), which can provide relief, although symptom reduction is often inadequate (1,2). The small market size for alphaviral arthritides generally, and the rapid, sporadic, and unpredictable nature of outbreaks caused by certain alpha-viruses like CHIKV (1), present major hurdles for the development and deployment of virus-specific interventions such as vaccines (7) or antibodies (8). Thus, therapeutic drug treatment will likely remain the only option for most patients.

A number of new treatments for CHIKV disease have been investigated. Unfortunately, chloroquine treatment was ineffective in human trials (9). Findings from murine studies suggest that interferon-α (IFN-α) treatment is only effective if given before infection (10), and that anti–tumor necrosis factor (anti-TNF) agents may, if administered during the acute phase, exacerbate disease (11). Steroid treatment (12) and steroid treatment combined with NSAIDs (13) appeared to provide clinical benefit in patients with RRV disease and in those with CHIKV disease, respectively; however, steroid treatment may not be appropriate for these self-limiting diseases, given the potential side effects (12). The most active area of antirheumatic drug development is for RA (14–16). The market size is large, with an estimated RA prevalence of ∼1% among adult white populations in Europe and the US. Importantly, there is also a growing awareness that ideal new therapeutic agents for RA should not compromise antipathogen immunity (16), a consideration that is also clearly important in treatments of viral arthritides (3).

Although there are some similarities between alphaviral arthritides and RA (3), there are also key differences. RA is generally a progressive disease that results in bone and cartilage erosion, has a female:male prevalence ratio of ∼2.5:1, is more common in people older than age 65 years, is associated with specific HLA–DRB1 alleles (17), involves neutrophils, and is believed to be driven by autoimmune T cells (primarily Th17) and B cells specific for citrullinated proteins (18). In contrast, alphaviral arthritides are generally self-limiting, do not normally show erosive changes, have a female:male prevalence ratio of ∼1.2:1, are rare in children, are characterized by mononuclear infiltrates (2,4,5,10), have no established HLA association, and have T and B cell responses that are generally directed at CHIKV
antigens (5,10). Results from murine studies also suggest that the contribution of T and B cells to alphaviral rheumatic disease may be limited (19–21).

We recently developed an experimental model of CHIKV arthritis in adult wild-type mice that mimics many of the features of human CHIKV disease, including a 4–5-day viremia that is followed by arthritic disease (10). In the present study, we report data from a microarray analysis of foot tissues from mice following infection with 2 CHIKV isolates, an Asian isolate from the 1960s and an isolate from the recent epidemic on Reunion Island (10). These data were used to identify a consensus CHIKV arthritis gene signature, which was compared with the signatures previously reported for patients with RA (22) and for collagen-induced arthritis (CIA), a mouse model of RA (23). There was a surprisingly significant overlap between the CHIKV arthritis and the RA gene signatures, and the CHIKV arthritis and the CIA gene signatures. These results provide hope that at least some of the antiinflammatory drugs and biologic agents being developed for RA might also be effective in the treatment of alphaviral arthritides.

MATERIALS AND METHODS

Ethics statement

All animal experiments complied with the Australian National Health and Medical Research Council guidelines. The animal studies were approved by the Queensland Institute of Medical Research animal ethics committee.

Preparation of viral isolates

The Reunion Island CHIKV isolate (strain LR2006-OPY1) and Asian CHIKV isolate (GenBank accession no. FJ457921) were prepared as described previously (10). The virus preparations had undetectable endotoxin and mycoplasma contamination (10).

Infection of mice and monitoring of arthritis

C57BL/6 mice were supplied by the Animal Resources Centre in Perth, Australia. Mice deficient in MHCII (MHCIIΔ/Δ) (24) and mice deficient in interferon-γ (IFNγ−/−) (JR3288 B6.129S7-Ifng/J; The Jackson Laboratory), each bred on the C57BL/6 background; mice were bred at the Queensland Institute of Medical Research. Groups of mice were inoculated with the Asian or Reunion Island isolates of CHIKV as described previously (10). Briefly, the mice were inoculated with CHIKV (log10 50% cell culture infectivity dose [CCID50] 10^4, in 40 μl RPMI 1640 supplemented with 2% fetal calf serum [FCS]) by shallow subcutaneous injection into the top of each hind foot, toward the lateral side in the metatarsal region, with injection directed toward the ankle. Arthritis was monitored by determining the extent of hind foot swelling using digital calipers, measured as the height and width of the metatarsal area of the hind foot. Results, presented as the mean value for each group, are expressed as the percentage increase in foot height × width for each foot, compared with the same foot on day 0.

Preparation of RNA samples

At the indicated times postinfection, mice were killed and 2 feet from each of 3 mice were removed, cut open with a scalpel, and placed in RNAlater (Ambion) for 24 hours at 4°C and then stored at −70°C. Two feet from each of 3 mice were also mock infected with 40 μl RPMI 1640 (supplemented with 2% FCS), and after 6 hours, the animals were killed and the feet were removed. For uninfected controls, 2 feet from each of 6 mice were left uninfected and treated as above.
Prior to RNA extraction, the feet were transferred into a tube containing 1.5 ml of TRIzol reagent (Invitrogen) and two 5-mm stainless steel balls (Qiagen), and the tissue was then homogenized (3 times 2 minutes, at 25 Hz) using a Qiagen TissueLyser. The resultant TRIzol solutions were centrifuged at 12,000g for 10 minutes to remove debris, and the RNA was purified from the supernatants according to the manufacturer’s instructions (Invitrogen). For each isolate and time point, equal amounts of RNA from each foot were pooled.

**Microarray experiments and analyses**

Microarray experiments were performed as described previously (25), using Mouse Gene 1.0ST arrays (Affymetrix). Probe sets for all samples were normalized using the robust multiarray average (RMA) algorithm, which includes global background adjustment and quantile normalization. Probe sets that do not represent a known transcript, according to the Affymetrix annotation, were discarded from further analyses. Principal components analysis (PCA) was performed using all of the remaining probe sets.

To identify differentially expressed genes at a given time point for each CHIKV isolate, we used a previously described statistical framework (26). This method uses an iterative procedure to perform robust estimation of the null hypothesis, which assumes that the gene expression background difference is normally distributed, allowing the identification of differentially expressed genes as outliers (at a level of significance of $P < 0.05$ and false discovery rate [FDR] of <10%). Differentially expressed genes were identified relative to the 6-hour mock-infections (see Supplementary Figure 1A, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131).

Gene networks and functional relationships were analyzed with Ingenuity Pathway Analysis (IPA; Ingenuity Systems) and the Bioinformatics Database for Annotation, Visualization, and Integrated Discovery (available at http://david.abcc.ncifcrf.gov/). Publicly available microarray raw data files (CEL files) from studies of patients with RA (GSE1919) and mice with CIA (GSE13071) were downloaded from the GEO data repository (available from the National Center for Biotechnology Information [NCBI] at http://www.ncbi.nlm.nih.gov/geo), and the expression data were normalized using the RMA algorithm. Based on the Affymetrix annotations for each gene probe set, the probe sets representing the same gene were collapsed by taking the one with the highest median value across all samples. For comparisons between human and mouse studies, human genes from the GSE1919 RA study were converted into mouse homologs utilizing the NCBI Homolo-Gene annotation (available at http://www.ncbi.nlm.nih.gov/homologene). Gene set enrichment analysis (GSEA) (27) was performed to determine whether CHIKV gene signatures (gene sets) were enriched in the RA and CIA expression data sets. The parameters used in the GSEA were the weighted enrichment statistic and Signal2Noise metric, with 1,000 permutations.

**RESULTS**

**Correlation between gene expression data and disease manifestations**

A total of 10 RNA samples were used to analyze gene expression. The samples of pooled RNA were derived from the following groups: 1) 6 feet (3 mice) infected with the Reunion Island or Asian CHIKV isolates, harvested on days 0.25, 1, 3, and 7 postinfection, 2) 6 feet (3 mice) from mock-infected mice, harvested at 6 hours (0.25 days) postinfection, and 3) 12 feet (6 mice) from mice left uninfected as controls.

Results from PCA using all 28,310 annotated probe sets showed a consistent pattern of clustering of expression data from the Asian and Reunion Island CHIKV–infected feet at each time point (Figure 1A). In addition, expression data from the mock-infected and
uninfected control mice also grouped together, indicating that the inoculation process had only a limited impact on gene expression (Figure 1A).

To evaluate whether the array data reflected the observations previously described for this model of CHIKV arthritis, the up-regulated genes in the feet infected with Reunion Island and Asian CHIKV isolates were subjected to Gene Ontology (GO) and IPA analyses. Genes associated with activation of macrophages, activation of phagocytes, and movement of phagocytes were all significantly up-regulated in the infection groups, with the number of genes in these pathways (Figure 1B) and their fold increases in expression relative to those in mock-infected feet (Figure 1C) progressively rising until day 7. (Days 6–7 postinfection represents the time of peak arthritis in this model, when foot swelling and inflammatory infiltrates are at their maximum [10].) A prominent feature of alphavirus-induced rheumatic disease (1), also seen in a CHIKV monkey model (4) and in the CHIKV mouse model of arthritis used herein (10), is a prolific infiltrate of monocytes and macrophages. Genes associated with inflammation were also found to be up-regulated in the infection groups (Figures 1B and C). Consistent with this, we have previously observed an up-regulation of the pro-inflammatory mediators TNF, IFN-γ, and monocyte chemotactic protein 1 (MCP-1) in the feet of infected mice, determined by real-time reverse transcription–polymerase chain reaction (10). An increase in natural killer (NK) cell–associated genes was also revealed in the pathway analyses (Figures 1B and C). We have previously shown that the cellular infiltrate in the arthritic feet of these mice contains ∼16% NK cells, as determined by fluorescence-activated cell sorting (10).

Although serum IFN-α/IFN-β levels (as measured by bioassay) reach peak levels on days 1–2 in this model (10), we observed a continued up-regulation of genes associated with the type I IFN response in the infected feet of these mice (Figures 1B and C), which likely reflects the ongoing viral replication in the feet. Previously, we found that virus titers of ∼6 log_{10} CCID_{50}/gm were still present on day 7 postinfection (10). On day 7, the feet infected with Reunion Island CHIKV also showed more swelling and displayed a more pronounced macrophage infiltrate than that seen in the feet infected with Asian CHIKV (10). This is consistent with our findings of a significantly higher mean fold up-regulation in gene expression (in all of the aforementioned pathways) in Reunion Island CHIKV–infected feet compared to Asian CHIKV–infected feet on day 7 (P < 0.001 by paired t-test) (Figure 1C).

A significant number of genes were also down-regulated after CHIKV infection (Figure 1B, bottom row). The most dramatic down-regulation of gene expression occurred in Reunion Island CHIKV–infected feet on day 1 postinfection (1,009 genes down-regulated). Analysis of these down-regulated genes using GO, IPA, and InnateDB only consistently revealed pathways associated with muscle tissue. Muscle-associated genes were also substantially down-regulated in Asian CHIKV–infected feet, but this occurred on day 3 postinfection, and more so on day 7 postinfection of muscle (Figure 1C). In a recent study, we showed that skeletal muscle cells are infected in this arthritis model (28). Since alphavirus infection of mammalian cells usually results in shut-down of host cell transcription within 4–20 hours (29) and apoptosis in 1–2 days (30), the down-regulation of muscle genes may be due to direct infection of the muscle cells. CHIKV infects a number of other cell types, including fibroblasts, endothelial cells, and monocyte/macrophages, and therefore the large number of down-regulated genes in Reunion Island CHIKV–infected feet on day 1 may reflect infection of these cells. This contention was supported by the increase in apoptotic cells observed in Reunion Island CHIKV–infected feet on days 2–3 postinfection (see Supplementary Figure 1B, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). Viral titers were also 10-fold higher in Reunion Island CHIKV–infected feet than in Asian CHIKV–infected feet on day 1 (10), which is consistent with the greater number of down-regulated genes observed in
Comparison of genes differentially expressed in Asian CHIKV– and Reunion Island CHIKV–infected feet

To analyze the differences between the 2 CHIKV isolates, differentially expressed genes from Asian CHIKV–infected feet and Reunion Island CHIKV–infected feet were compared at each time point. We found that a number of genes differentially expressed in Reunion Island CHIKV–infected feet at one time point were differentially expressed in Asian CHIKV–infected feet at another time point (see Supplementary Table 1, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). This was especially true for genes down-regulated on day 1. Of the 1,009 down-regulated genes in Reunion Island CHIKV–infected feet on day 1, only 68 were also down-regulated in Asian CHIKV–infected feet at this time point. However, of these 1,009 genes, 304 on day 3 and 458 on day 7 were down-regulated in Asian CHIKV–infected feet (as shown in Supplementary Table 1).

We thus determined the number of up- or down-regulated genes in Reunion Island CHIKV–infected feet at each time point that were also up- or down-regulated, respectively, at any time point in Asian CHIKV–infected feet. Figure 2 shows the number of differentially expressed genes shared between Reunion Island CHIKV–infected feet and Asian CHIKV–infected feet at each time point (white outlined bars), and the number of differentially expressed genes in Reunion Island CHIKV–infected feet at a given time point that were also differentially expressed in Asian CHIKV–infected feet at any time point (black bars). This process also revealed that genes that were uniquely differentially expressed in the Reunion Island CHIKV–infected feet (Figure 2, blue bars) generally had a lower fold change in expression (mean ± SD values shown in blue in Figure 2) postinfection when compared with genes that were shared between the Asian and Reunion Island CHIKV infection groups (Figure 2, black bars and mean ± SD values shown in black). The mean fold change in gene expression in the unique differentially expressed genes (values shown in blue in Figure 2) was also close to the cutoff values for up- or down-regulated genes (see Figure 2 legend).

The consensus gene sets identified on day 7 postinfection (time of peak arthritis) were used as the gene signature of CHIKV arthritis. The gene sets comprised 872 up-regulated genes and 374 down-regulated genes on day 7 in the Reunion Island CHIKV–infected feet that were also up- and down-regulated in the Asian CHIKV–infected feet at any time point (Figure 2, black bars) (see also Supplementary Table 1, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131).

Similarity between the gene expression signature from CHIKV-infected arthritic feet and synovial tissue from human patients with RA

To determine how similar the gene signature of CHIKV arthritis was to that seen in RA, the up- and down-regulated CHIKV arthritis gene sets were compared with a publicly available microarray data set from a study of RA patients and healthy subjects (22). GSEA software (27) was used to determine whether the rank-ordered list of genes in the RA study (i.e., genes ranked by their fold change in expression in RA patients relative to healthy individuals [22]) was enriched in genes derived from the CHIKV arthritis gene sets. The nominal P value and FDR are provided for each comparison (i.e., genes up-regulated during CHIKV arthritis and RA, and genes down-regulated during CHIKV arthritis and RA) (Figure 3A).
Despite the different etiology, the different species, and the different tissue preparations (i.e., whole feet in the CHIKV arthritis model versus synovial tissue samples in the RA study), there was a significant enrichment of up-regulated genes in CHIKV arthritis among the genes that were up-regulated in RA patients (Figure 3A, left panel). An enrichment of down-regulated genes, approaching significance, was also seen among the genes that were down-regulated in RA patients (compared with healthy controls) (Figure 3A, right panel). Furthermore, the degree of up-regulation of the 282 genes identified by the GSEA (which compared CHIKV arthritis and RA) appeared to correlate with the RA inflammation severity score (Figure 3B) (the list of the 282 up-regulated genes is provided in Supplementary Table 2, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). The correlation and significance of this finding was determined using the “distance to health” score (31) (Figure 3C).

GSEA was undertaken, in the same manner as described above, using data sets from 2 other RA studies. For one of these data sets (GSE24060), there was a significant enrichment of up-regulated genes when compared with the CHIKV arthritis gene set ($P = 0.018, \text{FDR} = 0.021$), even though the source tissue for the RA study was peripheral blood mononuclear cells rather than synovial tissue. In the other data set (GSE12021), there was no significant enrichment ($P = 0.17, \text{FDR} = 0.017$) (see Supplementary Figure 1C, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). However, 10 of 12 patients in the latter study (GSE12021) were taking prednisolone, whereas only 1 of 8 patients in the RA study (22) analyzed in Figure 3 was taking steroids.

All of these analyses suggest a high degree of similarity between the inflammatory arthritis gene signatures of CHIKV arthritis and RA. Of note, no enrichments were seen (FDR >25%) when either the RA (GSE1919) or the CIA (GSE13071) data sets were compared with differentially expressed genes reported for blood of monkeys infected with Venezuelan equine encephalitis virus, an alphavirus not associated with arthritis (32) (results not shown).

**Similarity of the gene expression signature from CHIKV-infected arthritic feet and synovial tissue from mice with CIA**

GSEA was used to compare the CHIKV arthritis gene sets with a publicly available microarray data set from a study of mouse CIA, which compared the gene expression in synovial tissue samples from mice with severe CIA and healthy mice (23). As in the study of human RA, the GSEA showed that there was a significant enrichment of up- and down-regulated genes when the CHIKV arthritis gene set was compared with the gene set from CIA (Figure 4). The results of these analyses suggest that there is a high degree of concordance in the inflammatory arthritis gene signatures between CHIKV arthritis and CIA.

**Gene network analysis**

To determine the gene networks and pathways shared between CHIKV arthritis and human RA, and between CHIKV arthritis and mouse CIA, the genes identified as enriched in the GSEA (Figures 3A and 4) were interrogated using IPA. A significance cutoff of $P < 0.01$ was used, and pathways containing fewer than 4 genes were excluded. When the up-regulated genes were analyzed, a large list of pathways emerged, which were grouped into a series of themes (Figure 5A). This process illustrated that CHIKV arthritis and RA, as well as CHIKV arthritis and CIA, all share up-regulated genes that are associated with T cells, autoimmunity, antigen presentation, NK cells, innate sensing, monocyte/macrophages, apoptosis, B cells, chemokines/cytokines, and complement (Figure 5A and Supplementary...
Table 2, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). A group of other pathways was also identified (Figure 5A); however, the majority of genes in these other pathways were also present in the aforementioned pathways (see Supplementary Table 2). Gene network analyses of the enriched down-regulated genes for both RA and CIA only revealed pathways associated with muscle (see Supplementary Table 2).

GSEA was used to compare the CHIKV arthritis gene set with expression data sets from 8 nonarthritic conditions, including autoimmune diseases and viral infections. Of these conditions, 2 showed significant enrichment (yellow fever vaccination and influenza infection). However, the pathways shared between CHIKV arthritis and these 2 viral data sets were lower in number and more restricted to antiviral pathways (Supplementary Figure 1C, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). Thus, although CHIKV arthritis shares pathways with other viral infections, the numbers of pathways shared between the arthritic tissue from CHIKV and RA/CIA were relatively higher and were more broadly distributed across multiple themes.

The large and highly significant set of shared T cell–associated pathways identified by GSEA (Figure 5A) was surprising, as previous reports suggested that adaptive immune responses have little or no effect on alphaviral rheumatic disease (19–21). The shared cytokine pathways between CHIKV and RA were IFN, interleukin-4 (IL-4), TWEAK, IL-10, TNF, IL-15, granulocyte–macrophage colony-stimulating factor, IL-8, and lymphotoxin B. The overlap of genes involved in IL-17 signaling only just reached significance, with a log(P value) of 2.1 (Supplementary Table 2, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131), perhaps consistent with the view that this cytokine in RA and CIA is involved in bone erosion (33), which is not generally seen during CHIKV arthritis.

Role of T cells and IFNγ in CHIKV arthritis

CHIKV infections appear to generate robust virus-specific CD4 T cell responses in mice and humans (5,10), and CD4 T cells play a role in RA (34,35). However, studies in young mice infected with RRV suggested that T cells are not important in alphaviral pathology (19–21). To determine the contribution of CD4 T cells to arthritic disease in the adult mouse model of CHIKV infection used herein, we infected mice lacking all 4 of the classic murine MHCII genes (MHCIIΔ/Δ) (24). Foot swelling after CHIKV infections was significantly reduced in MHCIIΔ/Δ mice compared with wild-type mice on days 6 and 7 (Figure 5B), the time of peak of arthritic disease (10). CD4 T cells thus do appear to be important players during alphaviral arthritic disease. (In both MHCIIΔ/Δ mice and Foxn1nu mice, the acute CHIKV viremia was controlled, despite the lack of CD4 T cells [results not shown].)

The cytokine pathway that showed the most significant overlap between CHIKV arthritis and RA was the IFN signaling pathway (log(P value) = 11.2), which includes genes associated with IFNα/IFNβ signaling (important for antiviral responses) and IFNγ signaling (see Supplementary Table 2, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). Th1 CD4 T cells that secrete IFNγ are involved in RA (34,35), and NK cells and IFNγ-secreting CD4 T cells are present in CHIKV-infected mice (10). Serum IFNγ levels are also elevated in CHIKV-infected monkeys (4) and in patients with CHIKV disease (5,36). To ascertain the contribution of IFNγ to CHIKV arthritic disease, IFNγ–/– mice were infected with CHIKV, and foot swelling was measured at various time points after infection. The degree of foot swelling was significantly reduced in IFNγ–/– mice compared to wild-type mice on days 6 and 7.
(Figure 5B). (The viremia was unaffected in IFN γ−/− mice [results not shown].) These findings suggest that IFN γ significantly contributes to CHIKV arthritis.

**DISCUSSION**

Herein we show a remarkable similarity between the gene signatures of CHIKV arthritis and RA, with the concordance between the signatures appearing to increase with the severity of the RA inflammation score. The similarity is particularly surprising given the different species (mouse and human), the distinct disease etiologies, and the different tissues sampled (feet versus synovial biopsy tissue). The arthritis genes identified by GSEA analysis of the CHIKV arthritis and RA data sets were associated with a wide range of canonical pathways that involved T cells, autoimmunity, antigen presentation, NK cells, innate sensing, monocyte/macrophages, apoptosis, B cells, cytokines/chemokines, and complement. An important conclusion from this analysis is that drugs and biologic agents being developed for RA that target these pathways may therefore also find utility in the improved treatment of CHIKV and perhaps other alphaviral arthritides.

Some recent studies have suggested that CHIKV disease may lead to RA (37–39). However, these studies may simply have identified patients with RA who were also infected with CHIKV (1,40). Given the high attack rate of CHIKV disease (38% in Reunion Island [2]) and an RA prevalence of ~1% in the general population, such patients are likely to be common in epidemic areas. Whether the rate of RA is ultimately found to be higher in CHIKV epidemic areas remains to be established, and any studies attempting to address this issue would have to account for increased detection rates simply arising from a greater general awareness of rheumatic disease after a CHIKV epidemic. Currently, there is no good evidence to support the notion that viral arthritides lead to autoimmune disease (3), and the current study should not be interpreted as providing insights into etiology. Nevertheless, a small number of elderly patients with CHIKV disease do appear to develop protracted RA-like illness (5,39), and in these patients, treatment with methotrexate seems to be effective (5).

The broad spectrum of overlapping pathways between CHIKV and RA opens up a range of potential new treatment options for alphaviral arthritides. For instance, a number of drugs that modify T cell activities are being developed (14–16,18), which may also work for alphaviral arthritides. A range of cytokines and chemokines are being targeted in a quest to develop drugs for autoimmune diseases such as RA (41). Unfortunately, human studies of cytokine/chemokine production following CHIKV infection have, so far, not provided an overly coherent picture (5,36,42), complicating any choice of optimal targets. Observations from small-scale trials have suggested that anti-IFN γ antibodies might be effective against RA (43), and the results from our studies suggest that targeting IFN γ may be an effective approach for ameliorating CHIKV arthritis. This observation is consistent with the findings from previous studies showing that anti-IFN γ antibodies can ameliorate RRV disease in young mice (44).

Targeting induction of MCP-1/CCL2, a chemo-kine strongly up-regulated in CHIKV disease (4,10, 36,42), with binding was shown to be effective in mouse models of CHIKV disease (45). Although this cytokine is also up-regulated in RA, trials of a CCL2 receptor-blocking antibody in RA have been disappointing (46). Drugs blocking complement activation are being developed for a number of diseases, including RA (47), with evidence from studies of mice suggesting that complement activation also contributes to alphaviral disease (48).
Promotion of apoptosis of T cells and fibroblast-like-synoviocytes may emerge as another useful approach in the treatment of RA (49). Whether interventions that modulate apoptosis would be of benefit for alphaviral arthritides remains to be established (30). Although IFNα and B cell responses are likely to play important roles in the pathologic processes of RA (18,50), targeting these as a treatment strategy for alphaviral arthritides may be considered too risky. Chronic CHIKV arthritis is likely due to the persistence of replication-competent virus (4), and the presence of IFNα/IFNβ antibodies is clearly important for antiviral activity (10).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The Supported by a grant from the International Alliance Program of the Government of Queensland for collaboration between the Australian Centre for Vaccine Development and Emory Vaccine Center. Dr. Nakaya was recipient of a travel award from the Australian Centre for Vaccine Development.

References


Figure 1.
Global gene expression changes induced by chikungunya virus (CHIKV) infection. A, Unsupervised principal components analysis (PCA) was used to assess clustering of expression data obtained from feet of mice days (d) 0.25, 1, 3, and 7 after infection with Reunion Island or Asian CHIKV isolates, feet of mock-infected mice, and feet of uninfected control mice. Expression data from all 28,310 annotated probe sets were used for this analysis. B, Pathways that were significantly enriched with differentially expressed genes in Reunion Island CHIKV-infected and Asian CHIKV-infected feet were identified. Values in boxes are the numbers of up- or down-regulated genes in each pathway at each time point. The total number of up- or down-regulated genes is shown in bold in parentheses. Pathways were obtained from the Gene Ontology and Ingenuity databases; the total number of genes in each pathway is given in parentheses next to the pathway description. C, Temporal expression patterns of the same pathways shown in B. Lines show the mean fold-change in expression of genes within each pathway for both CHIKV isolates at each time point. The number of genes used to determine the means is shown in parentheses. Asterisks indicate statistically significant difference (paired t-test P value <0.001 using all the genes in parentheses) between the Reunion Island CHIKV-infected and Asian CHIKV-infected feet. NK = natural killer; IFN = interferon.
Figure 2.
Comparison of differentially expressed genes in Asian chikungunya virus (CHIKV)–infected and Reunion Island CHIKV-infected feet. The number of differentially expressed genes in Reunion Island CHIKV-infected feet at each time point postinfection is shown in black plus blue bars. The white outlined bars represent the number of these genes that were also differentially expressed in Asian CHIKV-infected feet at the same time point. The black bars represent the number of genes that were differentially expressed in Reunion Island CHIKV-infected feet at the indicated time point that were also differentially expressed in Asian CHIKV-infected feet at ANY time point (Shared). The blue bars represent the number of genes that were uniquely differentially expressed in Reunion Island CHIKV-infected feet at the indicated time point (Unique). The numbers above the bars represent the mean ± SD fold change in gene expression in Reunion Island CHIKV-infected feet for unique (blue) or shared (black) genes. For probe sets that represent the same gene, the highest differential expression value was used for this calculation. Cutoff values for up-regulation on days 0.25, 1, 3, and 7 postinfection were 1.7, 1.5, 1.5, and 1.6, respectively, in the Reunion Island isolates and 1.7, 1.5, 1.4, and 1.6, respectively, in the Asian isolates, while cutoff values for down-regulation at these time points were 1.7, 1.5, 1.5, and 1.6, respectively, in the Reunion Island isolates and 1.7, 1.5, 1.4, and 1.6, respectively, in the Asian isolates.
Figure 3.
Comparison of chikungunya virus (CHIKV) arthritis and rheumatoid arthritis (RA) gene signatures. A, GSEA analysis showing a statistically significant enrichment of up- (left panel, 282 genes in “core enrichment” out of 465 genes) and down- (right panel, 68 genes in “core enrichment” of 187 genes) regulated genes from CHIKV arthritis in the up- and down-regulated genes, respectively, identified in RA (22). The CHIKV arthritis genes (Figure 2, black bars, day 7) are listed in Supplementary Table 1, on the Arthritis & Rheumatism website at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131 (22). B, A heatmap showing the relative expression levels of the 282 up-regulated genes in the CHIKV arthritis gene set that were enriched in RA patients. Columns represent the 5 control, and 5 RA
samples in the study (22) and their respective inflammation scores. The 282 genes are listed in Supplemental Table 2. C, Positive correlation between “Distance to health” and inflammation scores. The “Distance to health” (black bars) was calculated as described (30). Briefly, the number of standard deviations from the mean expression of healthy samples was determined for each of the 282 genes in B. The score was calculated by summing these standard deviations (where SD from the mean > 2) of all 282 genes. The box outlined in blue (left) shows the Pearson’s correlation and significance of the association (by 1-tailed t-test). FDR = false discovery rate.
Figure 4.
Comparison of chikungunya virus (CHIKV) arthritis and mouse collagen-induced arthritis (CIA) gene signatures. GSEA analysis showing a statistically significant enrichment of up- (left panel, 437 genes in “core enrichment” out of 751 genes) and down- (right panel, 173 genes in “core enrichment” out of 328 genes) regulated genes from CHIKV arthritis in the up- and down-regulated genes, respectively, identified in CIA (23). The CHIKV arthritis gene sets were the same as those used in Figure 3. FDR = false discovery rate.
Figure 5.
Gene pathway analyses, and role of T cells and cytokines in chikungunya virus (CHIKV) arthritis. A, Ingenuity Pathway Analysis of the 282 up-regulated genes identified by GSEA (core enrichment) in Figure 3A (CHIKV and rheumatoid arthritis [RA]) (left panel) and Ingenuity Pathway Analysis of the 437 up-regulated genes identified by GSEA (core enrichment) in Figure 4 (CHIKV and collagen-induced arthritis [CIA]) (right panel). The canonical pathways identified are grouped into themes, and the log(P value) is plotted for each pathway. As individual genes can appear in multiple pathways, the total number of unique genes within each theme is shown to the right of the bars. Of the 101 genes in the “Other” pathway for RA, all but 22 are present in the preceding themes. Of the 106 genes in...
the “Other” pathway for CIA, all but 26 are present in the preceding themes. The pathways and the genes are listed in Supplementary Table 2, on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131. B, A reduction in foot swelling is seen in mice deficient in MHCII (MHCII ΔΔ) and mice deficient in interferon-γ (IFN-γ –/–). Wild-type, MHCII ΔΔ, and IFN-γ –/– mice were infected with CHIKV, and foot swelling was measured at various time points after infection. In wild-type mice, histologically confirmed arthritis peaks on days 6/7 postinfection, which manifests as readily measurable foot swelling (10). Bars show the mean ± SEM. *P < 0.001 for wild-type mice (n = 14–30 feet) versus MHCII ΔΔ mice (n = 20 feet) on days 6 and 7, by t-test; *P = 0.011 on day 6 and *P = 0.003 on day 7 for wild-type versus IFN-γ –/– mice (each n = 10 feet), by t-test.