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ORIGINAL ARTICLE

Allograft Inflammatory Factor-1 Links T-Cell Activation, Interferon Response, and Macrophage Activation in Chronic Kawasaki Disease Arteritis

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Background. Kawasaki disease (KD) is widely viewed as an acute arteritis. However, our pathologic studies show that chronic coronary arteritis can persist long after disease onset and is closely linked with arterial stenosis. Transcriptome profiling of acute KD arteritis tissues revealed upregulation of T lymphocyte, type I interferon, and allograft inflammatory factor-1 (AIF1) genes. We determined whether these immune responses persist in chronic KD arteritis, and we investigated the role of AIF1 in these responses.

Methods. Gene expression in chronic KD and childhood control arteries was determined by real-time reverse-transcriptase polymerase chain reaction, and arterial protein expression was determined by immunohistochemistry and immunofluorescence. Allograft inflammatory factor-1 small-interfering ribonucleic acid macrophage treatment was performed to investigate the role of AIF1 in macrophage and T lymphocyte activation.

Results. Allograft inflammatory factor-1 protein was highly expressed in stenotic KD arteries and colocalized with the macrophage marker CD68. T lymphocyte and interferon pathway genes were significantly upregulated in chronic KD coronary artery tissues. Alpha interferon-induced macrophage expression of CD80 and major histocompatibility complex class II was dependent on AIF1, and macrophage expression of AIF1 was required for antigen-specific T lymphocyte activation.

Conclusions. Allograft inflammatory factor-1, originally identified in posttransplant arterial stenosis, is markedly upregulated in KD stenotic arterial tissues. T lymphocyte and type I interferon responses persist in chronic KD arteritis. Allograft inflammatory factor-1 may play multiple roles linking type I interferon response, macrophage activation, and antigen-specific T lymphocyte activation. These results suggest the likely importance of lymphocyte-myeloid cell cross-talk in the pathogenesis of KD arteritis and can inform selection of new immunotherapies for clinical trials in high-risk KD children.

Keywords. allograft inflammatory factor-1; arteritis; Kawasaki disease.

The model of Kawasaki disease (KD) arteritis as solely an acute, self-limited arteritis derives from pathologic interpretations of light microscopy studies of autopsied KD cases from the 1970s. This model proposed that inflammatory cell infiltration of coronary arteries occurred in the first few weeks of illness with resolution of inflammation within 2–3 months after onset [1, 2]. However, most KD deaths in these series occurred in the first several weeks after the onset, and evidence of chronic arterial inflammation was reported in the small number of KD cases in which death occurred several months after the onset [3]. Intravenous gammaglobulin therapy has significantly reduced the prevalence of coronary artery abnormalities in KD patients [4]; however, some patients still develop significant coronary artery disease resulting in death or need for transplant because of delayed diagnosis and/or failure to respond to therapy [5]. We described 3 linked KD vasculopathic processes—acute self-limited necrotizing arteritis, subacute/chronic arteritis, and luminal myofibroblastic proliferation—that explain the severe outcomes that can be associated with KD [5]. Our ability to identify the presence of chronic arteritis and associated luminal myofibroblastic proliferation in KD tissues was enabled by study of ~20 KD cases who died or had surgery more than 3 months after KD onset, more such cases than were available for study in the 1970s [1, 2]. The apparent link between chronic arteritis and luminal myofibroblastic proliferation leading to stenosis raises the question as to whether a trial of prolonged immunomodulatory therapy for KD children with persisting arteritis should be considered. Presently,

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KD patients with arteritis receive only short-course immunomodulatory therapy.

Our recent report of the transcriptome of acute KD coronary arteritis at a median of 4 weeks after the onset showed marked upregulation of cytotoxic T lymphocyte and interferon (IFN) responses. We also noted significant upregulation of allograft inflammatory factor-1 (AIF1) [6]. Allograft inflammatory factor-1 was initially cloned from the heart tissue of rats with posttransplant vasculopathy [7]. Expression of AIF1 has been correlated with the development of human cardiac allograft vasculopathy [8]. In this study, our objective was to determine whether the T lymphocyte and type I IFN responses previously detected at a median of 4 weeks after KD onset would remain upregulated in tissues of patients with persistent arteritis at ≥5 months after the onset. We also determined whether expression of AIF1 protein was upregulated in KD stenotic arteries, as has been observed in posttransplant arterial stenosis [8], and investigated the role of AIF1 in macrophage and T lymphocyte activation and IFN response.

METHODS

Patients, Controls, and Tissues

The study was approved by the Institutional Review Board (IRB) of Ann & Robert H. Lurie Children's Hospital of Chicago. Tissues from outside institutions were sent to us deidentified and coded and were therefore IRB exempt. Informed consent was obtained for cases in which the identity of the patient was known to any member of the research team, and the samples were coded to preserve confidentiality. The pathologic features of the coronary arteries in the KD patients are described in our prior studies [5, 6]. Demographic and treatment information on the KD patients and their corresponding case numbers in our pathologic study are given in (Table 1). Childhood controls had normal coronary artery histology; their demographic characteristics and diagnoses are given in (Table 2). Coronary arteries from KD patients were individually embedded at autopsy/transplant, whereas control epicardial coronary arteries were microdissected from myocardial tissue blocks and re-embedded before sectioning. For studies of immune gene expression in chronic coronary arteritis and for AIF1 immunohistochemistry, we tested coronary artery tissues obtained from patients at least 5 months after the onset of KD. Three additional cases in which significant luminal myofibroblastic proliferation (LMP) was present at 2.5 to 5 weeks after onset were assayed using the LMP gene array (Table 1). For comparison of AIF1 staining in KD arteries to human and murine posttransplant vasculopathy, tissue sections of explanted heart were obtained from a 14-year-old girl who underwent a second retransplant for posttransplant vasculopathy, and sections of mouse heart were obtained 27 days after allogeneic transplant.

Ribonucleic Acid Isolation

Ribonucleic acid (RNA) was isolated from formalin-fixed, paraffin-embedded (FFPE) KD and control tissue sections using the RNeasy FFPE kit (QIAGEN, Valencia, CA) according to manufacturer's instructions, except that tissue lysis was performed at 55°C for 1 hour. Single-strand complementary deoxyribonucleic acid (cDNA) was synthesized from 300 ng of extracted RNA using the QIAGEN/SA Biosciences cDNA synthesis kit according to the manufacturer's instructions.

Real-Time Polymearse Chain Reaction

Real-time reverse-transcriptase polymearse chain reaction (RT-PCR) array assays were performed using the RT2 Pre-AMP cDNA synthesis kit, RT2 qPCR primers, and RT2 SYBR green master mix (QIAGEN). Reactions using primers for the housekeeping gene HPRT1 (QIAGEN) were performed as an internal control, and primers for human genomic DNA were used as a quality control measure. Kawasaki disease and control samples were considered to be of good quality if the real-time PCR threshold cycle [C(t)] values for human genomic DNA were at least 3 C(t) higher than for the housekeeping gene HPRT1 (ie, at least 8-fold more RNA than DNA was present in the sample) and the RNA housekeeping gene HPRT1 C(t) was less than 32.

Table 1. Demographic and Clinical Data on Kawasaki Disease Patients Whose Coronary Artery Tissues Were Assayed in This Study

Case	Age (Yr)	Time Since Onset (Yr of Death or Surgery)	Gender	Ethnicity	KD Therapy	Arrays	AIF1 IHC	Case No. in Ref. 5
1	6	5 mo (2008)	M	White	None*	Immune		26
2	5	7.5 mo (2008)	F	White	IVIG, steroid, infliximab	Immune, Custom	x	27
3	1.5	10 mo (1998)	M	Black	None	Immune, Custom	x	28
4	3	2 yr (2000)	M	Asian	Unknown*	Immune, Custom	x	33
5	1	Unknown (2002)	M	Unknown	Unknown*	Immune, Custom	x	37
6	19	16 yrs (2008)	M	Hispanic	None	Immune		34
7	2	7 mo (2011)	F	White	None	Immune, Custom	x	NA, Ref. 6
8	0.3	3–4 week (2006)	M	White	IVIG, steroid	Custom		11
9	0.3	5 week (2005)	M	Unknown	IVIG, steroid	Custom		18
10	0.9	2.5 weeks (1997)	M	White	None	Custom		4

Abbreviations: AIF1, allograft inflammatory factor 1; IHC, immunohistochemistry; IVIG, intravenous gammaglobulin; KD, Kawasaki disease; NA, not applicable; Ref., reference.
*,transplant.

For differential expression analysis, we used the comparative C_T method [9], where C_T is defined as the PCR cycle at which the fluorescent signal of the reporter dye crosses an arbitrarily placed threshold. Arrays were performed on each sample, and results were normalized to the internal HPRT1 housekeeping gene on each array. A moderated 2-sample *t* test [10] was used to compare differential expression measured by ΔC_T, where $\Delta C_T = (C_T$ gene of interest $- C_T$ internal control). We controlled for false discovery rate to account for multiple testing under dependency using adjusted *P* values with ≤.1 considered significant [11].

Immunohistochemistry

Immunohistochemistry was performed on FFPE tissue sections as previously described [12, 13], using antigen retrieval and an avidin-biotin-horseradish peroxidase system (Vectastain Elite ABC kit; Vector, Burlingame, CA). Rabbit anti-human AIF1 antibody validated by the Human Protein Atlas project [14] was from Sigma-Aldrich (St. Louis, MO) (HPA049234, 1:200 dilution).

Immunofluorescence

Immunofluorescence staining of FFPE tissues was performed using antigen retrieval as for immunohistochemistry, but primary antibodies for AIF1 were used at 1:10, and secondary antibody was goat antirabbit AlexaFluor 488 (Invitrogen, Carlsbad, CA). Colocalization for CD68 (monocyte/dendritic cell/macrophage), CD3 (T lymphocyte), and smooth muscle actin (smooth muscle cells) was assessed using mouse antihuman antibodies from Dako (Carpinteria, CA) (all at 1:10 dilution) and goat antimouse AlexaFluor 568 (Invitrogen).

Macrophage Cultures

Murine bone marrow cell cultures were differentiated into bone marrow-derived macrophages using Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 20% L929-conditioned media. The cells were stimulated with IFN-α (100 U/mL) or IFN-γ (100 IU/mL) for 24 hours, and expression of AIF1 messenger RNA (mRNA) was determined by real-time PCR assay in unstimulated cells and those treated with IFN-α or IFN-γ. In another experiment, 3×10^5 macrophages were plated in 96-well U-bottom plates on day 7 of culture and treated with either control or AIF1 small-interfering RNA (siRNA) for 48 hours using the Lipofectamine RNAiMAX (Invitrogen) transfection reagent according to the manufacturer's instructions. Macrophages were then stimulated with either IFN-α (100 U/mL; PBL Assay Science) or IFN-γ (100 IU/mL; Invitrogen) for 24 hours, and expression of CD80 and major histocompatibility complex (MHC) class II were determined by flow cytometry.

T Lymphocyte-Macrophage Cocultivation Assay

Interferon-stimulated control or AIF1 siRNA-treated murine bone marrow-derived macrophages were stimulated with IFN-α or IFN-γ as described above. After macrophage activation, the cells were loaded with OT-II peptide (ovalbumin peptide 323–339, 10 μM) for 2 hours before the addition of 2×10^5 carboxyfluorescein succinimidyl ester (CFSE)-labeled OT-II T lymphocytes, which are specific for ovalbumin antigenic peptide 323–339 in the context of I-A b [15]. Macrophages and OT-II cells were cocultured with the addition of interleukin (IL)-2 (5 ng/mL) to the media for an additional 72 hours and then analyzed by fluorescence-activated cell sorting for T-cell proliferation.

RESULTS

Allograft Inflammatory Factor-1 Protein Is Highly Expressed in Kawasaki Disease Chronic Arteritis/Stenotic Arterial Lesions

Our transcriptome analysis revealed that AIF1, which has been associated with arterial stenosis resulting from posttransplant vasculopathy [8], was upregulated in acute KD arterial lesions with luminal myofibroblastic proliferation [6]. To visualize AIF1 protein and its tissue distribution in chronic KD arteritis, we performed immunohistochemistry on 5 KD cases with significant arterial stenoses using a validated antibody [14]. In all of 5 cases, many AIF1-positive cells were observed in the stenotic chronic arteritis/luminal myofibroblastic proliferation tissue mass (Figure 1A, B, and C), similar to findings in posttransplant vasculopathy (Figure 2A and B). We also detected AIF1 expression in stenotic coronary arteries in a murine model of posttransplant vasculopathy (Figure 2C). Childhood control coronary arteries did not show AIF1-positive cells (Figure 1D), and isotype control antibody stains of the tissues were negative. This is the first demonstration of the extensive expression of AIF1 protein in KD coronary arteritis with associated stenosis.

Allograft Inflammatory Factor-1-Positive Cells in Kawasaki Disease Stenotic Arteritis Lesions Are CD68 Positive

To determine the cell type expressing AIF1, immunofluorescence colocalization studies were performed on cases 3 and 5. Allograft inflammatory factor-1 colocalized with the macrophage cell marker CD68 (Figure 3), but not with smooth muscle actin or with CD3 (Supplemental Figure 1).

Increased Expression of T Lymphocyte Activation-Induced Genes in Persisting Kawasaki Disease Arteritis Revealed by Polymearse Chain Reaction Array Studies

Light microscopic findings in KD coronary artery tissues [5] led us to hypothesize that persistent arteritis was an activated T lymphocyte process, potentially induced by AIF1 and other factors. To determine whether there was upregulation of genes associated with T lymphocyte activation, we concurrently tested coronary artery RNA samples (n = 7 chronic KD arteritis cases and n = 7 childhood controls) on quantitative RT-PCR arrays specific for T lymphocyte activation-induced

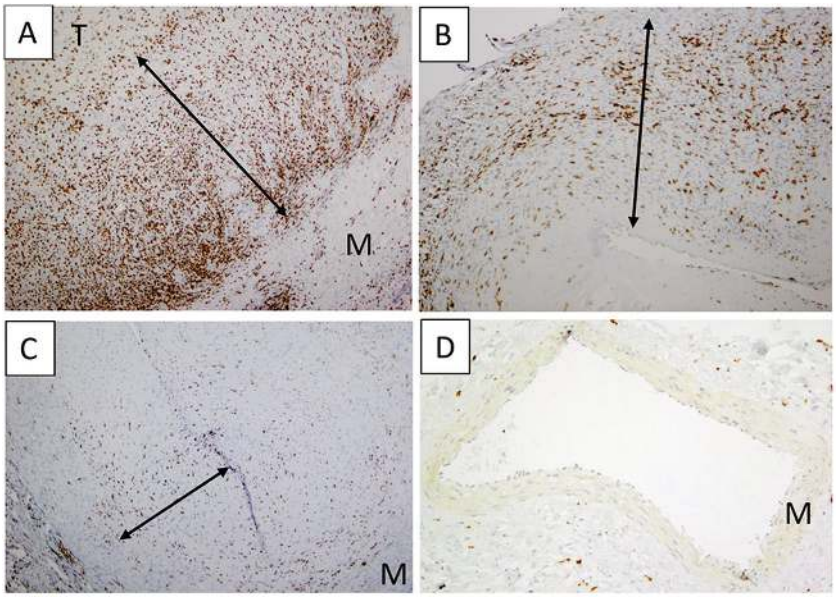

Figure 1. Allograft inflammatory factor-1 (AIF1) is highly expressed in Kawasaki disease (KD) coronary arteries with chronic arteritis and coronary artery stenosis. Numerous AIF1-positive cells (brown) are observed in the luminal myofibroblastic proliferation tissue mass (double-sided arrows) in (A) KD case 5, where overlying thrombus (T) has further contributed to arterial occlusion; (B) KD case 4, where a slit-like lumen is observed at the inferior tip of the arrow; and (C) KD case 3, where there is virtually complete luminal occlusion at the right tip of the arrow. (D) Childhood control C7 negative for AIF1-positive cells in the coronary artery wall, although adventitial tissue has scattered positive cells. (A–C) ×4 objective; (D) ×10 objective. M, media.

genes (QIAGEN). Of 84 genes evaluated (Supplemental Table 1), 24 were significantly upregulated (Table 3). CD80, a protein found on activated B cells and macrophages that provides a costimulatory signal necessary for T-cell activation and survival, exhibited over 90-fold increase in expression in KD coronary artery tissues compared with childhood control coronary arteries. CD4, found on T-helper cells, monocytes, macrophages, and dendritic cells, exhibited 50-fold higher expression in persisting KD coronary arteritis. Additional cytokines and chemokines such as IL10, IL3, CCL5, IL9,

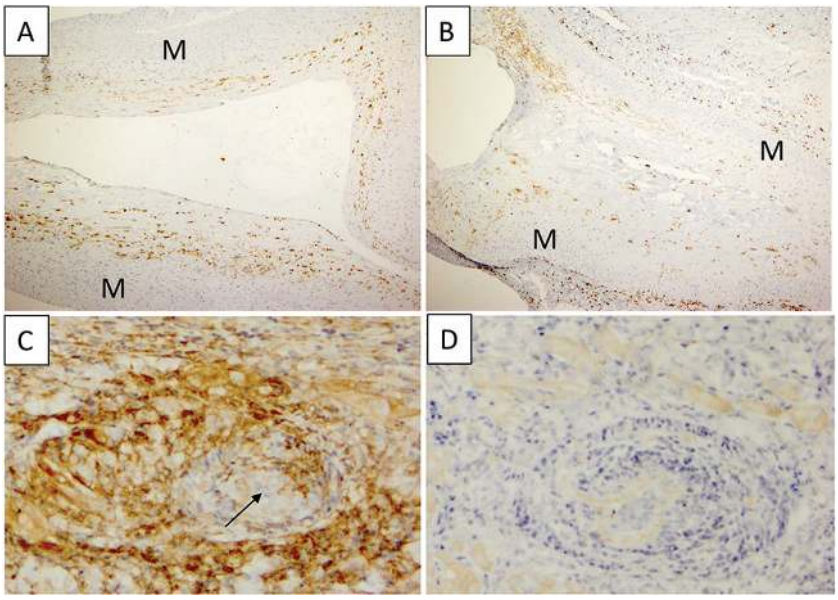

Figure 2. Allograft inflammatory factor-1 (AIF1) is highly expressed in human and murine coronary arteries with posttransplant vasculopathy (PTV). (A and B) Human coronary arteries with PTV show numerous AIF1-positive cells (brown) in thickened intima-media, and remaining lumen of stenotic coronary artery in B is at upper left. (C) Stenotic murine coronary artery with PTV has many AIF1-positive cells, arrow points to obliterated lumen. (D) Isotype control antibody staining of section adjacent to that shown in C is negative. (A and B) ×4 objective; (C and D), ×10 objective. M, media.

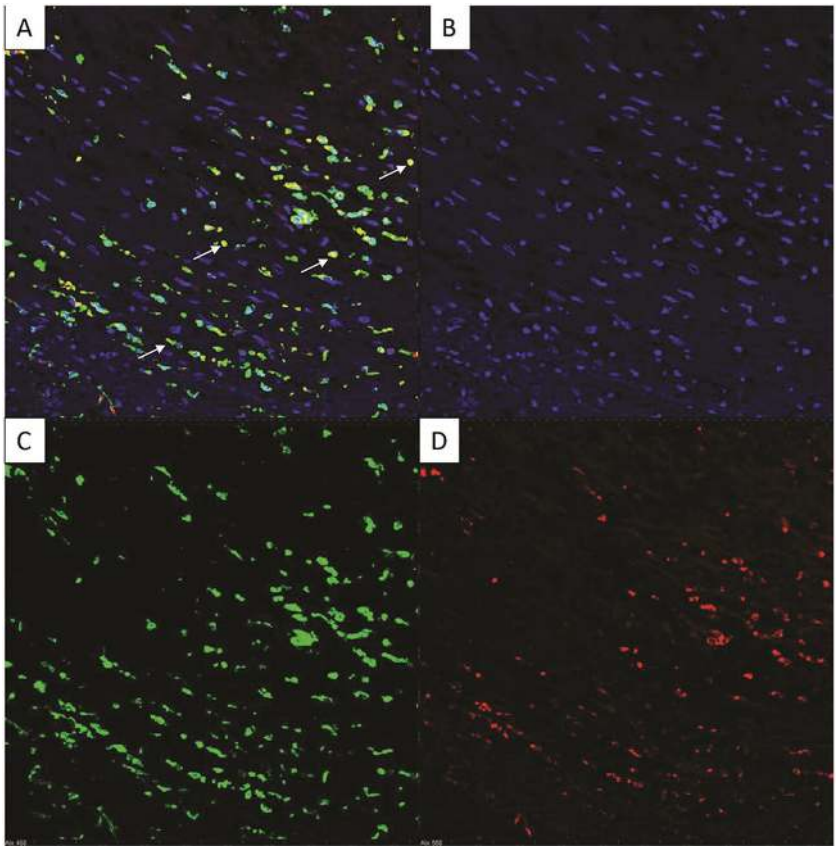

Figure 3. Allograft inflammatory factor-1 (AIF1) colocalizes with myeloid marker CD68 in stenotic Kawasaki disease (KD) coronary artery by immunofluorescence. (A) Overlay image showing many colocalizing cells (white arrows); (B) 4′,6-diamidino-2-phenylindole; (C) AIF1 (green); (D) CD68 (red). KD case 3, ×20 objective.

and IL25 were 10- to 20-fold higher in expression in the KD compared with the control coronary arteries. These results complement our light microscopy [5, 12] and transcriptome profiling studies [6] demonstrating an activated T lymphocyte response in KD coronary artery tissues and provide new evidence of prolonged arterial immune activation ≥5 months after disease onset.

Increased Expression of Interferon Receptor and Interferon Response Genes Revealed by Quantitative Real-Time Polymearse Chain Reaction

We hypothesized that chronic arteritis at least 5 months after KD onset would be associated with a persistent IFN response, similar to the IFN response signature that we previously identified in acute KD coronary arteries [6]. Therefore, we tested the KD and childhood control coronary artery RNA

Table 2. Demographic and Clinical Data on Childhood Control Patients With Normal Coronary Artery Histology Whose Tissues Were Assayed in This Study

Case	Age	Gender	Diagnosis	Arrays	AIF1 IHC
C1	2 years	M	Liver failure, seizures, Marfan, Alpers syndrome	Immune	x
C2	11 months	M	Hypoplastic left heart, respiratory syncytial virus infection	Immune, Custom	x
C3	5 months	M	Pneumococcal meningitis, disseminated intravascular coagulation	Immune	x
C4	10 months	M	Prematurity, neurologic devastation secondary to Serratia meningitis, chronic lung disease	Immune	
C5	12 days	F	Meconium aspiration, pulmonary hemorrhage	Immune	
C6	9 years	M	Developmental delay, seizures, fever	Immune	
C7	4 years	F	Small bowel obstruction, pneumonia	Immune	x
C8	2 months	F	Prematurity, cerebral hemorrhage, bronchopulmonary dysplasia, bronchopneumonia	Custom	
C9	1 months	M	Congenital myopathy, subdural and liver hematomas	Custom	x
C10	2 days	M	Left diaphragmatic hernia	Custom	x
C11	4 days	F	Congenital sacrococcygeal teratoma	Custom	

Abbreviations: AIF1, allograft inflammatory factor-1; IHC, immunohistochemistry.

Table 3. Genes Upregulated in Chronic Kawasaki Disease Coronary Arteries on Immune Gene Expression Real-Time Polymerase Chain Reaction Array

Gene	Fold Change	Adjusted *P* Value
CD80	97.2	5.6e-04
CD4	53.3	.009
HAVCR2	38.2	.070
IL10	20.2	.033
IL3	19.1	.083
CCL5	16.6	.002
IL9	14.2	.063
IL25	13.8	.070
CCR5	11.7	.009
LAT	9.8	.009
SPP1	9.5	.040
IL2RA	8.1	.030
IFNG	7.2	.073
IRF4	7.0	.070
PTPRC	6.1	.070
IL18	4.6	.070
MAF	4.4	.063
CD28	4.3	.083
EBI3	3.9	.073
STAT1	3.3	.070
NFATC1	3.3	.070
IL15	3.1	.070
IL13RA1	2.3	.084

samples using arrays focused on IFNs and relevant receptors (QIAGEN). Of 84 genes evaluated (Supplemental Table 1), 15 were upregulated including IFN responsive genes MX1, OAS1, and IFIT2, each of which showed greater than 9-fold upregulation (Table 4). These results confirm and extend our observations on the upregulation of IFN-responsive genes in KD arteritis [6].

Table 4. Genes Upregulated in Chronic Kawasaki Disease Coronary Arteries on an Interferons and Receptors Real-Time Polymerase Chain Reaction Array

Gene	Fold Change	Adjusted *P* Value
IFI44L	42.1	.061
IL2RG	20.7	.009
MX1	18.9	.018
CSF2RA	12.2	.024
IL5RA	12.1	.069
IFNG	10.4	.039
OAS1	9.9	.018
IFIT2	9.9	.039
IFI30	8.0	.018
IL10RA	7.5	.039
IFI6	6.6	.064
IRF5	6.5	.031
IL15	5.5	.039
IFI44	4.6	.085
IRF7	3.6	.098

Additional Selected Growth Factor and Apoptosis Genes Were Not Dysregulated in Kawasaki Disease Coronary Artery Tissues With Luminal Myofibroblastic Proliferation

To test the hypothesis that dysregulation of selected growth factors and/or apoptosis genes is present in the luminal myofibroblastic pathologic proliferative process, we developed a custom PCR array that included 87 common growth factor and apoptosis genes, including members of the transforming growth factor-β family, several fibroblast growth factors, vascular endothelial growth factor A, platelet-derived growth factors, caspases, and members of the BAX/BAD/BCL2 families, some of which were previously postulated to be involved in the development of stenotic arterial lesions in KD [16] (Supplementary Table 1). None of the 87 genes were significantly dysregulated in KD coronary arteries with luminal myofibroblastic proliferation. These results indicate that members of these gene families are likely not as prominently dysregulated in chronic KD arteritis as T lymphocyte and type I IFN-induced genes.

Allograft Inflammatory Factor-1 Expression Is Induced in Macrophages by Interferon-α and Interferon-γ

Stimulation of bone marrow-derived macrophages by IFN-γ resulted in increased expression of AIF1 mRNA (Figure 4A), as previously reported for other macrophage cell lines [7]. It is interesting to note that stimulation by IFN-α also resulted in increased expression of AIF1 mRNA (Figure 4A).

Interferon-α-Induced Expression of Macrophage Activation Markers Is Allograft Inflammatory Factor-1 Dependent

Because AIF1 was induced by IFNs, we hypothesized that IFN-induced expression of macrophage activation markers MHC class II and CD80 was AIF1 dependent. The expression of MHC class II on bone marrow-derived macrophages stimulated with IFN-α was significantly reduced by AIF1 siRNA treatment, and the expression of CD80 was modestly reduced by this treatment (Figure 4B). Stimulation of these cells by IFN-γ resulted in the expected upregulation of CD80 and MHC class II, but AIF1 siRNA treatment did not result in a significant reduction of their expression (data not shown).

Allograft Inflammatory Factor-1 Expression in Macrophages Is Required to Induce Antigen-Specific T-Cell Proliferation

Macrophages are professional antigen-presenting cells, so we postulated that macrophage expression of AIF1 in KD might contribute to antigen-driven T-cell activation. To test this idea, bone marrow-derived murine macrophages treated with control or AIF1-specific siRNA were pulsed with OT-II to induce proliferation of OT-II-specific T cells as demonstrated by CFSE dilution assay. Allograft inflammatory factor-1 expression in macrophages was required for antigen-specific T lymphocyte activation, because AIF1 siRNA knockdown abrogated this effect (Figure 4C). Although AIF1 expression has previously been associated with T-cell activation [17, 18], to our knowledge

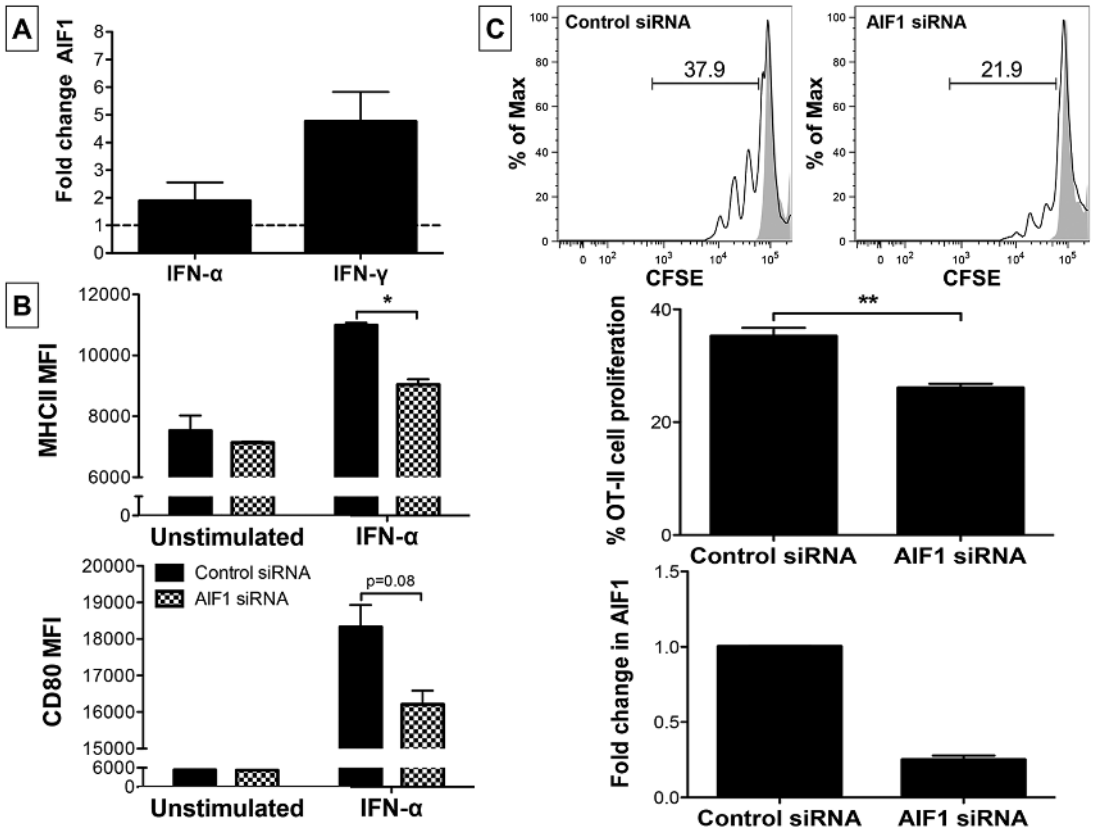

Figure 4. Allograft inflammatory factor-1 (AIF1) is required for interferon (IFN)-α-dependent macrophage activation and for antigen-specific T lymphocyte proliferation. (A) Allograft inflammatory factor-1 messenger ribonucleic acid (RNA) is induced by IFN-α and IFN-γ in bone marrow-derived macrophages. (B) Interferon-α-induced major histocompatibility complex (MHC) class II expression in bone marrow-derived macrophages is significantly decreased with AIF1 small-interfering RNA (siRNA) treatment, and CD80 expression is modestly reduced. (C) Allograft inflammatory factor-1 siRNA treatment significantly reduces antigen-specific (ovalbumin) T lymphocyte activation as determined by carboxyfluorescein succinimidyl ester (CFSE) dilution. Reduction in expression of AIF1 with siRNA treatment is shown at bottom right. *$P < .05$; **$P < .01$.

this is the first study to show that macrophage AIF1 is required for antigen-dependent T-cell activation. These studies imply a role for AIF1 in antigen-specific T-cell proliferation and expression of macrophage activation markers, and that some of these functions are IFN-dependent, providing evidence of a potential role for AIF1 in the pathogenesis of chronic KD arteritis.

DISCUSSION

It is now clear that a subset of children diagnosed with KD suffer from complications that are unexpected for an acute self-limited arteritis. These KD complications include cases of persistent coronary artery inflammation and luminal obliteration at autopsy months to years after onset [5, 19–22], enlarging coronary artery aneurysms months to years after onset [23–27], and myocardial infarction many years after coronary artery luminal diameters appeared normal by angiography [28, 29]. However, all of these complications are consistent with the pathology of chronic arteritis and luminal myofibroblastic proliferation, as described in our extensive pathologic study [5]. The prevalence of chronic vasculitis and luminal myofibroblastic proliferation among KD patients with coronary artery complications is presently unknown. It was recently reported that KD patients with progressive coronary artery dilation 2 months or later after onset have more severe outcomes than those in whom dilation does not progress after this time point [27]. In a preliminary study, we reported upregulation of CD84, which facilitates T-cell immune responses, in coronary arteries from patients who died or underwent heart transplant 5 months or longer (up to 2 years) after KD onset [30].

Allograft inflammatory factor-1 is a protein that binds actin and calcium, is induced by cytokines and IFN, and may play a role in linking inflammation to cellular proliferation [31]. Because AIF1 has been implicated in proliferative arteriopathies such as posttransplant vasculopathy [8, 17, 32, 33] and systemic sclerosis [34], and because we found its gene expression to be significantly upregulated in KD arterial tissues at a median of 4 weeks after onset [6], we examined its protein expression in KD lesions with chronic arteritis and arterial stenosis. We found AIF1 protein to be significantly upregulated in

chronic KD arteritis tissues in myeloid cells expressing CD68. We also identified upregulated T lymphocyte and type I IFN responses in these tissues. Gene polymorphisms resulting in a decrease in negative regulation of T lymphocyte responses have been associated with the development of KD and coronary artery abnormalities [35, 36]. Persistence of type I IFN responses has been reported in chronic viral infections [37, 38] and in autoimmune diseases [39]. Whether the persistence of chronic arteritis in KD is due to continued antigenic stimulation by a human or microbial antigen or whether it is a result of persistent immune dysregulation due to a genetic or other cause is presently unknown. We found that AIF1 was upregulated in macrophages after treatment with IFN-α, that expression of macrophage activation markers by IFN-α was AIF1 dependent, and that antigen-specific T lymphocyte proliferation in vitro was AIF1 dependent. These findings suggest the importance of AIF1 in linking persisting type I IFN responses with T lymphocyte proliferation in KD chronic arteritis.

Ongoing macrophage-T lymphocyte interactions could provide an inflammatory mileu that stimulates smooth muscle cell-derived myofibroblasts to proliferate and cause arterial stenosis. Upregulation of AIF1 expression has been observed in coronary allograft vasculopathy [8], systemic sclerosis [40], and, in this report, KD, 3 conditions in which arterial stenosis results in significant adverse clinical outcomes. We also found AIF1 to be upregulated in coronary arteries in a murine model of posttransplant arterial stenosis, suggesting that this model may be useful in identifying the role and mechanism of AIF1 in proliferative arteriopathies. Further investigation of this protein's role in arterial stenosis resulting from diverse inflammatory etiologies is warranted.

CONCLUSIONS

Our study demonstrates the complexity of immune responses in severely affected KD patients with chronic arteritis, and it demonstrates the likely importance of cross-talk between innate and adaptive immune cells in disease pathogenesis. This information can inform selection of new immune modulatory therapies for future multicenter clinical trials in very high-risk children with KD, including those with severe and persisting coronary artery abnormalities.

Supplementary Data

Supplementary materials are available at Journal of *the Pediatric Infectious Diseases Society* online.

Notes

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Potential conflicts of interest. All authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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