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CD43-Mediated IFN-γ Production by CD8⁺ T Cells Promotes Abdominal Aortic Aneurysm in Mice

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CD43 is a glycosylated surface protein abundantly expressed on lymphocytes. Its role in immune responses has been difficult to clearly establish, with evidence supporting both costimulatory and inhibitory functions. In addition, its contribution to disease pathogenesis remains elusive. Using a well-characterized murine model of elastase-induced abdominal aortic aneurysm (AAA) that recapitulates many key features of the human disease, we established that the presence of CD43 on T cells is required for AAA formation. Moreover, we found that IFN-γ-producing CD8⁺ T cells, but not CD4⁺ T cells, promote the development of aneurysm by enhancing cellular apoptosis and matrix metalloproteinase activity. Reconstitution with IFN-γ-producing CD8⁺ T cells or recombinant IFN-γ promotes the aneurysm phenotype in CD43⁻/⁻ mice, whereas IFN-γ antagonism abrogates disease in wild-type animals. Furthermore, we showed that the presence of CD43 with an intact cytoplasmic domain capable of binding to ezrin-radixin-moesin cytoskeletal proteins is essential for optimal in vivo IFN-γ production. These results further suggest that IFN-γ antagonism or selective blockade of CD43⁺CD8⁺ T cell activities merits further investigation for immunotherapy in AAA. The Journal of Immunology, 2013, 190: 000–000.

CD43 (leukosialin, sialophorin), a transmembrane glycoprotein highly expressed on various hematopoietic cells, has been extensively linked to various T cell activities and functions. A costimulatory function of CD43 was suggested following early observation that T cells from patients with Wiskott-Aldrich syndrome, an X-linked recessive immunodeficiency disorder, have altered or defective CD43 expression that accompanies defects in cytotoxicity and helper T cell functions (1, 2). However, CD43-deficient murine T cells were subsequently found to have increased proliferation to various stimuli and augmented cytotoxic T cell response, leading to the conclusion that CD43 negatively regulates T cell adhesion and activation (3).

The predominant model for CD43 function is that the large, negatively charged extracellular domain sterically impedes formation of an effective immunologic synapse. However, subsequent studies have demonstrated that the negative regulatory function of CD43 depends on its intracellular domain (4, 5). Phosphorylation of the CD43 cytoplasmic tail leads to its association with ezrin-radixin-moesin (ERM) cytoskeletal proteins and full T cell activation, whereas inhibition of CD43 interaction with ERM proteins results in decreased cytokine production (6–8). These findings are in agreement with reports showing that signaling through CD43 increases T-bet expression and inhibits GATA-3 gene transcription, predisposing T cells toward a Th1 lineage commitment and inducing IFN-γ expression (9–11). Conversely, CD43-deficient T cells preferentially differentiate into Th2 cells that produce high levels of IL-4, IL-5, and IL-13 (12). Congruent with these findings, CD43-deficient mice exhibit increased inflammation in Th2-mediated allergic airway diseases. In contrast, a preferential Th2 differentiation does not appear to clearly afford protection against Th1-mediated disease in nonobese diabetic mice or experimental autoimmune encephalomyelitis (EAE) (12), although conclusions regarding the exact contribution of CD43 to disease phenotype in the EAE model remain contradictory (13, 14).

Abdominal aortic aneurysm (AAA) is a common vascular disease characterized by transmural inflammation of the aortic wall tissues, excessive local production of proteolytic enzymes [matrix metalloproteinases (MMPs)] capable of degrading the extracellular matrix and of depletion or apoptosis of smooth muscle cells (SMCs), leading to the weakening and dilatation of the abdominal aorta (15). The inflammation in AAA is characterized by infiltration of the aortic wall with every type of leukocytes, including an abundance of lymphocytes (16). Greater than 50% of the lymphocytes recovered from AAA tissues are CD3⁺ T cells, including CD4⁺ and CD8⁺ T cells (17). Thus, elucidating the mechanisms by which T cells contribute to the inflammatory environment may further our understanding of the mechanisms that underlie the destructive process in AAA.

T cells in AAA tissues can express both Th1 cytokines (IL-2, IFN-γ) and Th2 cytokines (IL-4, IL-10). Although some reports suggest that Th1 cytokines are more consistently upregulated in large aneurysms (18–20) and expression of IFN-γ is increased in
the circulation and in tissues of patients with AAA, compared with controls (18, 20, 21), others suggest that a Th2 response predominates (22). Given the proposed costimulatory function of CD43, we wished to test the hypothesis that, by directing T cells toward a Th1 phenotype, CD43 promotes aneurysmal development. In this article, we show that CD43 deficiency confers complete resistance to elastase-induced AAA. In addition, we found that reconstitution with CD43-sufficient CD8+ T cells restores susceptibility to aneurysm development through an IFN-γ-dependent but perforin-independent mechanism.

Materials and Methods

Animals

Wild-type (WT) C57BL/6, C57BL/6-Ly5.1 (B6.SJL-Ptprc<sup>−/-</sup>Pten<sup>-/-</sup>/Boy), perforin<sup>−/−</sup> (C57BL/6-Pp<sup>−/−</sup>Pr<sup>−/−</sup>/Boy), IFN-γ<sup>−/−</sup> (B6.129S7-Ifng<sup>−/−</sup>129S7<sup>129S7</sup>), and CD8<sup>−/−</sup> (B6.129S2-Cd8a<sup>−/−</sup>) mice were obtained from The Jackson Laboratory. CD43<sup>−/−</sup> mice were generously provided by Dr. Jonathan Green (3, 4). CD43-FL and CD43-NGG transgenic (Tg) T cells were generously provided by Dr. Judy Cannon (8). All mice were kept in a pathogen-free condition at Washington University Specialized Research Facility (St. Louis, MO). All experiments were performed according to protocols approved by the Division of Comparative Medicine.

Elastase perfusion model of AAA

AAA was induced as previously described (23–25). Briefly, mice were anesthetized with 55–60 mg/kg i.p. sodium pentobarbital. A laparotomy was performed under sterile conditions. The abdominal aorta was isolated, and the preperfused aortic diameter (AD) was measured with a calibrated ocular grid. Temporary 7.0 silk ligatures were placed around the proximal and distal aorta to interrupt proximal flow. An aortotomy was created at the inferior ligature, using the tip of a 30-gauge needle, and the aortic lumen was perfused for 5 min at 100 mm Hg with a solution containing 0.145 U/ml elastase (St. Louis, MO). All experiments were performed according to protocols established at Washington University School of Medicine (26).

Immunohistochemistry

Immunohistochemistry for elastin and actin was performed as previously described (23–25). Briefly, mouse abdominal aorta was dissected, snap-frozen in OCT compound, and sectioned at 9 µm. Elastin was stained with Verhoef–van Gieson using an ACCUSTAIN Elastic Stain Kit (Sigma-Aldrich). SMC content was evaluated using an alkaline phosphatase–conjugated AB to α-smooth muscle actin (1:200 dilution; Sigma-Aldrich). Color was visualized using an Alkaline Phosphatase Substrate Kit (Vector Laboratories). Elastin degradation was graded on a scale of 1–4, as previously described (23–25): 1 < 25% degradation, 2 = 25–50% degradation, 3 = 50–75% degradation, and 4 = > 75% degradation. SMC actin content was also graded on a scale of 1–4 (23–25): 1 = < 25% loss, 2 = 25–50% loss, 3 = 50–75% loss, 4 = > 75% loss. Neutrophils, macrophages, and CD3<sup>+</sup> T cells were visualized with a biotinylated anti-Gr1 mAb (1:100 dilution; BD Biosciences), an anti-Mac-3 mAb (1:200 dilution; Cedarlane Laboratories), and anti-CD3 mAb (1:100 dilution; BD Biosciences), respectively. MMP-2<sup>+</sup> and MMP-9<sup>+</sup> cells were visualized with a rabbit polyclonal anti–MMP-2 Ab (0.2 µg/ml; Novus Biologicals) and an anti–MMP-9 mAb (2 µg/ml; R&D Systems). TUNEL assay was performed as recommended by the manufacturer (Millipore). Data presented were derived from six to nine serial cross-sections that spanned the entire abdominal aorta, with four to five aortas per genotype or treatment.

T cell isolation and adoptive transfer

CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> T cells were isolated from spleens and lymph nodes by negative selection using Miltenyi Biotec MACS microbeads. Cell purity was consistently > 95%. Isolated cells (7e6 for CD4<sup>+</sup> and CD8<sup>+</sup>, 1e7 for total CD3<sup>+</sup> T cells) were resuspended in 100 µl PBS and injected i.v. into mice on days 3 and 7 after elastase perfusion.

In vitro stimulation of T cells

CD8<sup>+</sup> T cells (2e5 per well) purified as above were activated with plate-bound anti-CD3 mAb (1 µg/ml; BD Biosciences) and soluble anti-CD28 mAb (5 µg/ml; BD Biosciences) in RPMI 1640 supplemented with 10% FBS in triplicates in 96-well plates. After 72 h, supernatants were harvested and assayed for IFN-γ and IL-10 by ELISA (R&D Systems).

In vivo competitive T cell recruitment

Lyst5.2 WT and CD43<sup>−/−</sup> T cells (1e7 each) were mixed at a 1:1 ratio and injected i.v. into congenic Ly5.1 mice on day 1 post elastase perfusion. Alternatively, CD3<sup>+</sup> T cells were differentially labeled with either CFSE (5 µM) or PKH-26 (2 µM), mixed at a 1:1 ratio (1e7 each) prior to transfer. On the indicated day, aortas were excised from below the renal arteries to above the femoral bifurcation, miniced, and digested, as previously described (23). Single-cell suspensions obtained were surface labeled for Ly5.1, Ly5.2, CD43, TCRβ, CD4, or CD8 (eBioscience) to distinguish donor cells (Lyst5.2<sup>+</sup> or CD43<sup>+</sup> Tg) from recipient cells (Ly5.1<sup>+</sup> or endogenous CD43<sup>+</sup> ) and analyzed by flow cytometry (FACSCalibur).

IFN-γ immunofluorescence image analysis

Cross-sections of aortic tissues (9 µm) were fixed in 4% paraformaldehyde, blocked in 8% BSA in PBS, and incubated with a goat polyclonal Ab to mouse CD43 (1:50 dilution; Santa Cruz Biotechnology), followed by rhodamine-conjugated donkey anti-goat Ab (1:100 dilution; Jackson ImmunoResearch) and FITC-conjugated IFN-γ (1:100 dilution; eBioscience). All images were visualized on a Nikon Eclipse fluorescence microscope and acquired with QCapture software using the same exposure time. Merged and single-color images were loaded into ImageJ software (http://rsb.info.nih.gov/ij/) for analysis. Threshold color of all images was set to the same hue, saturation, and brightness. With the brightness to filter the picture, positively stained areas were isolated by increasing the contrast between the color and background. This practice facilitates the selection of regions of interest (ROIs) by allowing ROIs to be selected easily with the wand tool. Double positive (CD43<sup>+</sup>IFN-γ<sup>+</sup>) cells were selected; single CD43<sup>+</sup> cells were deselected. The ROIs were measured on the unfiltered images, normalized to the positive area, and presented as integrated OD (IntDen). Data were obtained from six to eight fields per section and six to nine sections per aorta.

In situ zymography

Nonfixed, frozen sections (9 µm) of day 14 aortas were incubated with a fluorogenic gelatin substrate (DQ Gelatin) at 0.1 mg/ml; Molecular Probes) for 3 h at room temperature. For negative control, slides were incubated in the presence of 25 mM EDTA. The specific removal of essential divalent cations resulted in no detectable gelatinolytic activity.

All images were visualized on a Nikon Eclipse fluorescence microscope and acquired with QCapture software. The images were analyzed by ImageJ software, and image analysis software was normalized across the intensity of WT sections, which was set at 100%. Data represent six to nine sections per aorta, with four to five aortas per genotype or treatment.

Statistics

Comparisons between multiple groups were made by one-way ANOVA followed by Bonferroni’s posttest to compare all groups of data. Data are presented as the mean ± SEM. A p value < 0.05 was considered significant.

Results

CD43<sup>−/−</sup> mice are resistant to elastase-induced AAA

Transient porcine elastase perfusion of abdominal aorta is a widely used animal model of AAA that recapitulates many key features of the human disease histologically (27, 28). Immediately following intraluminal perfusion with elastase, we observed the same extent of AD dilatation in WT and CD43<sup>−/−</sup> mice (increase in AD of ~70%) (Fig. 1A, 1B). This AD has previously been shown to remain relatively stable until day 7 post elastase perfusion, after which there was a rapid increase in WT animals (23). Aneurysm is defined on day 14 as an increase in the AD of ≥100%, compared with the preperfused parameters (28). The absence of CD43 protected mice from aneurysm development (increase in AD of 91.8 ± 6.2% or 0.44 ± 0.03 mm in CD43<sup>−/−</sup> animals versus 144.8 ± 3.7% or 0.73 ± 0.1 mm in WT animals; p < 0.0001) (Fig. 1A, 1B). Histologic
analysis of day 14 aortas revealed severe fragmentation of the elastic fibers and depletion of SMC actin in WT animals, compared with the relatively well-preserved elastic lamellae and SMC actin content in CD43<sup>−/−</sup> animals (Fig. 1C–E; *p* < 0.0001).

Normal neutrophil recruitment but blunted chronic inflammatory response in CD43<sup>−/−</sup> mice

As CD43 is implicated in cellular trafficking (29–31), we first assessed the acute neutrophil response to elastase perfusion in CD43<sup>−/−</sup> mice on day 3. We found no difference between WT and CD43<sup>−/−</sup> mice in the number of neutrophils recruited to the aortic wall (Fig. 2A, 2B; *p* > 0.05). In contrast, histologic analysis of day 14 aortas revealed significantly fewer Mac-3<sup>+</sup> macrophages (*p* < 0.0001) and CD3<sup>+</sup> T cells (*p* < 0.0001) in CD43<sup>−/−</sup> mice (Fig. 2A, 2C, 2D). The lower CD3<sup>+</sup> T cell count reflects a decrease in both CD4<sup>+</sup> and CD8<sup>+</sup> T cells (Fig. 3). NK cell number, however, was relatively equivalent (946 ± 317 NK cells per WT aorta versus 985 ± 240 NK cells per CD43<sup>−/−</sup> aorta; *p* > 0.5). Consistent with a lower number of CD8<sup>+</sup> T cells, we observed ~60% reduction in the apoptotic index, as measured by TUNEL staining (Fig. 2A, 2E). Taken together, these results suggest that the absence of CD43 abrogates aneurysm development by dampening T cell and macrophage responses.

CD8<sup>+</sup> T cell reconstitution restores susceptibility to AAA development in CD43<sup>−/−</sup> mice

We next probed the contribution of T cells to aneurysm development. First, we reconstituted CD43<sup>−/−</sup> mice with total CD3<sup>+</sup> T cells. Reconstitution with WT CD3<sup>+</sup> T cells fully restored susceptibility to AAA in CD43<sup>−/−</sup> mice (increase in AD of 0.74 ± 0.02 mm WT T cell recipients versus 0.43 ± 0.03 mm CD43<sup>−/−</sup> T cell recipients; *p* < 0.001) (Fig. 4A). Next, we examined the contribution of CD8<sup>+</sup> versus CD4<sup>+</sup> T cells. We found that reconstitution with WT CD4<sup>+</sup> T cells did not induce AAA formation in CD43<sup>−/−</sup> mice.

![FIGURE 1.](image)

CD43<sup>−/−</sup> mice are resistant to elastase-induced AAA. WT and CD43<sup>−/−</sup> mice were perfused with elastase on day 0, and their AD was measured immediately post perfusion and again on day 14. Increase in AD was expressed in percentages (A) or millimeters (B). AAA [indicated by the red line in (A)] is defined as an increase in AD ≥ 100% over preperfusion parameters. Values represent mean ± SEM. The number of animals per genotype is indicated. (C) Staining for elastic fibers (upper panels) and actin content in SMCs (red stain, lower panels) showed extensive degradation of elastic fibers and depletion of SMC actin content in WT animals. Scale bar, 50 µm. Day 14 elastic fiber degradation (D) and SMC actin depletion (E) were graded on a scale of 1–4. Values represent mean ± SEM; *n* = 5 aortas per genotype. EL, Elastic lamellae; L, lumen. *p* < 0.0001.

![FIGURE 2.](image)

Absence of CD43 dampens the chronic inflammatory responses. (A) Sections of WT and CD43<sup>−/−</sup> aortas were stained for neutrophils (Gr-1<sup>+</sup> cells), macrophages (Mac-3<sup>+</sup> cells), T cells (CD3<sup>+</sup> cells), and apoptotic cells (TUNEL<sup>+</sup> cells). Scale bar, 100 µm. The number of Gr-1<sup>+</sup> cells (B), Mac-3<sup>+</sup> cells (C), CD3<sup>+</sup> cells (D), and TUNEL<sup>+</sup> cells (E) per aortic cross-section were enumerated. Values represent mean ± SEM; analysis was performed on 6–9 serial cross-sections per aorta; *n* = 4–5 aortas per genotype. *p* < 0.0001.
was accompanied by the relative preservation of elastic fiber integrity and SMC actin content (Fig. 5C, 5D) and a decrease in the number of Mac-3+ macrophages in the aortic wall tissues (p < 0.001) (Fig. 5E). Reconstitution of CD8+/ T cells with WT CD8+ T cells restored the AAA phenotype, whereas reconstitution with WT CD4+ T cells did not (Fig. 5A, 5B). CD8+ T cell transfer also led to an increase in the number of Mac-3+ macrophages localizing to the arterial wall (Fig. 5E), suggesting that CD8+ T cells are necessary for normal macrophage recruitment and/or activation during AAA development.

**Defective IFN-γ production by CD8+ T cells protects CD43−/− mice against AAA**

To determine whether a defect in T cell migration to the site of inflammation protects CD43−/− mice against AAA development, we used the allelic markers Ly5.1 and Ly5.2 (32) to distinguish donor and recipient T cells after adoptive transfer. Isolated CD8+ T cells (expressing Ly5.2) from WT and CD43−/− mice were mixed together (1:1 ratio) and transferred into congenic WT C57BL/6 mice expressing the allelic marker Ly5.1 on day 1 post elastase perfusion. On day 3, donor T cells (expressing Ly5.2) were recovered from abdominal aorta digest and analyzed for the expression of CD43. We found that CD43−/−CD8+ T cells migrated to the elastase-perfused aortic wall as efficiently as did WT CD8+ T cells (Fig. 6A), suggesting that the resistant phenotype cannot be explained by a defect in the initial recruitment of CD43−/− T cells to the elastase-perfused aorta.

In contrast, we found that CD43−/−CD8+ T cells had a significant defect in their ability to produce IFN-γ when activated in vitro with anti-CD3/CD28 mAb, whereas IL-10 production was normal (Fig. 6B). Given the previously reported role of IFN-γ in experimental AAA (33), we hypothesized that IFN-γ production by CD8+ T cells is required for aneurysm formation. To test this hypothesis, we injected elastase-perfused CD43−/− mice with 50,000 U of recombinant mouse IFN-γ on days 3, 6, 9, and 11 post perfusion. IFN-γ fully reconstituted the aneurysm phenotype in CD43−/− mice (Fig. 7A) and led to the characteristic elastic fiber fragmentation (Fig. 7B) and SMC actin depletion (Fig. 7C). In contrast, IFN-γ blockade with the mAb H22 (26, 34) abrogated AAA development in WT animals (Fig. 7A). To specifically determine the contribution of IFN-γ-producing CD8+ T cells in aneurysm development, we reconstituted CD43−/− mice with IFN-γ−/−CD8+ T cells. IFN-γ−/−CD8+ T cells failed to induce AAA in CD43−/− mice (Fig. 7A). These results strongly indicate that IFN-γ produced by CD8+ T cells is required for elastase-induced AAA.

**CD43 directs IFN-γ production in CD8+ T cells**

The above data support the idea that CD43+CD8+ T cells induce aneurysm formation by elaborating IFN-γ. To further elucidate how CD43 signals regulate IFN-γ production in T cells, we turned to a well-characterized CD43 Tg T cell model in which a mutation of the tripeptide KRR sequence within the intracellular domain of CD43 leads to a perturbation of its interaction with ERM proteins (5, 7, 8). Transfer of CD43-NGG Tg cells (those that express a form of CD43 in which the KRR sequence was mutated to NGG) (8) into elastase-perfused CD43−/− mice did not lead to AAA development (Fig. 8A). In contrast, elastase-perfused CD43−/− mice that received CD43-FL Tg cells (those that express the full-length WT CD43) developed aneurysm at the WT level (increase in AD of 0.44 ± 0.02 mm in CD43-NGG recipients versus 0.77 ± 0.3 mm in CD43-FL recipients; p < 0.001) (Fig. 8A). Histologic analysis of day 14 aorta confirmed that reconstitution with CD43-FL Tg cells led to severe fragmentation of elastic fibers and loss of SMC actin in CD43−/− recipient mice (data not shown).
Previous studies showed that perturbation of CD43 intracellular domain interaction with ERM proteins significantly decreases cytokine production (5, 7). Indeed, we confirmed that CD43-NGG Tg cells that migrated to the aortic wall of CD43−/− mice expressed ∼55% less IFN-γ when compared with CD43-FL Tg cells (Fig. 8B, Supplemental Fig. 1; p < 0.001). In addition, when we examined the CD43 Tg cells that migrated to the abdominal para-aortic lymph nodes of reconstituted mice, we also found a significant reduction in the percentage of CD43-NGG CD8+ T cells expressing IFN-γ as well as a decrease in the actual level of IFN-γ (Supplemental Fig. 2).

Because a previous report also showed that CD43-NGG cells have a defect in their ability to traffic to lymph nodes (8), we wanted to determine whether resistance to aneurysm development in CD43−/− mice that received CD43-NGG cells could be due to a defect in Tg cell recruitment. CD43-FL and CD43-NGG Tg cells were differentially labeled with fluorescent dyes, mixed at 1:1 ratio, and transferred to elastase-perfused recipients. Analysis of cells recovered from digested samples confirmed that the numbers of CD43-FL and CD43-NGG Tg cells recruited to elastase-perfused aortas at 20 h were equivalent (Fig. 8C). Taken together, the results above support the concept that the CD43 intracellular domain is required for the optimal production of IFN-γ and AAA genesis.

IFN-γ promotes cellular apoptosis in vivo

An important role proposed for CD8+ T cells in AAA is the mediation of SMC apoptosis, possibly through the perforin/granzyme granule exocytosis pathway (35). To determine the contribution of perforin in elastase-induced AAA, we examined perforin-deficient (Pfp−/−) mice (36, 37) and found that they were as susceptible to elastase-induced AAA as were WT animals (data not shown). Moreover, reconstitution of CD43−/− mice with Pfp−/−CD8+ T cells fully restored the AAA phenotype (Fig. 7A). Consistent with the susceptible phenotype, we found that reconstitution of CD43−/− mice with Pfp−/−CD8+ T cells led to significant elastic fiber degradation and SMC depletion (Fig. 7B, 7C). Moreover, we observed that reconstitution of CD43−/− mice with Pfp−/−CD8+ T cells or IFN-γ led to a significant increase in the apoptotic index (p < 0.001), whereas reconstitution with IFN-γ−/−CD8+ T cells did not (Fig. 7D). Overall, these results suggest that CD8+ T cells promote in vivo cellular apoptosis through an IFN-γ-dependent pathway, whereas perforin-dependent cytotoxicity is dispensable.

IFN-γ released by CD8+ T cells promotes MPP-producing macrophage recruitment

MMPs released by macrophages are thought to be responsible for local tissue destruction and the eventual aneurysmal dilatation (38). To understand whether IFN-γ modulates the local production and activity of MMPs, we first examined day 14 aortas from WT and CD43−/− mice. We observed that the number of Mac-3+ along with MMP-2+ and MMP-9+ cells infiltrating the aortic wall tissues was significantly lower in CD43−/− mice (p < 0.001) (Fig. 9A–D), as was the local gelatinase activity detected by in situ zymography, confirming that MMP activity was greatly attenuated in CD43−/− mice (p < 0.001) (Fig. 9E, 9F). Reconstitution of CD43−/− mice with WT CD8+ T cells or IFN-γ increased the number of Mac-3+, MMP-2+, and MMP-9+ cells (p < 0.001) (Fig. 9B–D) and enhanced local gelatinase activity (95% and 98% of WT level, respectively; p < 0.01) (Fig. 9F), whereas reconstitution of CD43−/− mice with IFN-γ−/−CD8+ T cells did not. In sum, these results suggest that IFN-γ produced by CD43+CD8+ T cells likely promotes the recruitment (or expansion) of MMP-producing macrophages and enhances the local MMP activity, both of which likely contribute to extracellular matrix degradation.
IFN-γ production that promotes AAA formation. (A) CD43−/− mice were perfused with elastase on day 0 and reconstituted with IFN-γ−/− CD8+ T cells, Pfp−/− CD8+ T cells, or administered recombinant mouse IFN-γ (rmIFN-γ) or anti-IFN-γ mAb. AD was measured on day 14. Values represent mean ± SEM. Day14 elastin degradation (B) and SMC actin depletion (C) were graded on a scale of 1–4. (D) TUNEL+ cells were enumerated per aortic cross-section. Values represent mean ± SEM; n = 5 aortas per treatment type. *p < 0.001 compared with CD43−/−.

Discussion

The findings in this article offer new insights into the role of CD43 in a relevant disease model that recapitulates key features of human AAA in many aspects. We showed that the presence of CD43 with an intact cytoplasmic domain capable of binding to ERM proteins is essential for IFN-γ production by CD8+ T cells and aneurysm formation. Moreover, we established an essential role for CD8+ T cells in AAA genesis. Although CD8+ T cells have long been implicated in the apoptosis of SMCs, a direct pathogenic role for these cells in AAA has not been established. Our data also suggest that IFN-γ elaborated by CD8+ T cells suffices to induce apoptosis in vivo, whereas perforin of the granule exocytosis pathway is dispensable. Conversely, blockade of IFN-γ activity abrogates aneurysm formation. IFN-γ released by activated CD8+ T cells promotes local tissue destruction through the recruitment or expansion of MMP-producing macrophages.

Although CD43 has been linked to many T cell activities (3, 9–11, 30, 39–41), its physiologic contribution to T cell functions in inflammation and diseases remains a point of debate. Previous studies have shown that the absence of CD43 results in a preferential Th2 differentiation and the exacerbation of Th2-mediated diseases (12). In addition, although a Th2-biased response suggests that CD43−/− mice should be protected against Th1-mediated diseases (such as EAE), the reports thus far present conflicting results, ranging from no differences in disease phenotype (12) to partial protection against disease induction (13, 14). In this article, we used the elastase-induced AAA model to establish that CD43 expression is required for CD8+ T cell activation and enhances IFN-γ production in vivo. A defect in IFN-γ production by CD43−/− T cells is not routinely described. However, CD43 interaction with ERM proteins is well documented and was previously mapped to the KRR sequence motif within the intracellular domain of CD43. Mutations of these residues perturb the interaction of CD43 with ERM proteins, blocking its accumulation at a pole complex distal to the immune synapse and diminishing the production of multiple cytokines in T cells, including IFN-γ (5–7). Moreover, a recent report demonstrates that the organization of this distal pole complex is required for optimal T cell activation, as conditional deletion of ezrin, in combination with small interfering RNA suppression of moesin, disrupts normal Ca2+ flux and phospholipase C-γ1 activation, and diminishes cytokine gene expression in T cells (42). Taken together, these results suggest that in the CD43-NGG Tg adoptive transfer model the ability of CD8+ T cells to induce the aneurysm phenotype is directly related to the interactions mediated by the cytoplasmic tail of CD43 with ERM proteins, the colocalization of which is required for optimal T cell activation and IFN-γ production (7, 42). Furthermore, we confirmed in vivo that the number of CD43−/− and CD43-NGG Tg cells initially recruited to the elastase-perfused aorta is comparable to that of WT and CD43-FL, thus ruling out a defect in T cell trafficking as the mechanism underlying disease resistance. The normal initial T cell recruitment further suggests that the reduction in T cell number observed on day 14 in CD43−/− aortic sections may reflect an imbalance in cell homeostasis or survival in the absence of CD43, consistent with previous studies suggesting that CD43 has an antiapoptotic function (43) or that CD43−/− T cells may be less capable of expansion (30).
The finding that only CD8+ T cells restore the aneurysmal phenotype in CD43
2/2 mice was unexpected. However, these data do not negate the previously established role for CD4+ T cells in CaCl2-induced AAA (33). Instead, our data suggest a hierarchal order with which T cells are activated and IFN-γ is produced during elastase-induced AAA development. The magnitude and rapidity with which activated CD8+ T cells initiate IFN-γ production in the early phase of the immune response likely explain their immunodominant role in further directing lineage commitment and expansion of that subset and surrounding leukocytes, including naive CD4+ T cells. This immunodominance of IFN-γ-producing CD8+ T cells during immune responses has previously been established in other model systems (44–46). However, the fact that CD432/2 mice have a slightly more resistant AAA phenotype (i.e., smaller AD on day 14 following elastase perfusion) than mice that are deficient only in CD8+ T cells suggests that CD43 expression on other leukocyte subsets, including CD4+ T cells, may indeed contribute to the overall AAA phenotype downstream of CD8+ T cell activation.

The observation that IFN-γ directs the inflammatory cascade in the elastase-induced AAA model is in agreement with a report by Xiong et al. (33). It stands, however, in stark contrast to other published studies. In the study by Shimizu et al. (47), the authors reported that aortic allografts transplanted into IFN-γ receptor null mice developed into severe AAA with a T2-predominant inflammation. Similarly, King et al. (48), using an apolipoprotein E− and IFN-γ−deficient model, showed that IFN-γ protects against aneurysm formation and rupture. Although we cannot reconcile these divergent findings, it should be noted that these models differ significantly from the elastase perfusion model used in our study. In addition, the Shimizu et al. and King et al. studies used models in which IFN-γ and IFN-γ receptor were absent throughout the animals’ development, whereas the IFN-γ deficiency in the CD432/2 mice is only relative. Thus IFN-γ may have a dynamic effect on T cells (and likely other leukocytes) that may be stimulatory or protective over the course of an immune response, depending on the level and the timing of its expression.

In summary, we have identified a robust phenotype for the CD43−/− mouse and established an important in vivo function for CD43-dependent regulation of IFN-γ production. Our results indicate that interactions mediated by the intracellular domain of CD43 in CD8+ T cells induce the production of IFN-γ that in turn orchestrates an inflammatory cascade, leading to the eventual aneurysmal degeneration. In addition, we have demonstrated for the first time, to our knowledge, a direct role for CD43+CD8+ T cells in the pathogenesis of AAA. Distinct populations of CD8+ T cells expressing different glycoforms of CD43 and possessing differential cytotoxic activity have been described in a model of murine intestinal inflammation (49). Whether these subpopulations of CD43+CD8+ T cells exist in humans and in AAA remains to be
determined. Nonetheless, these studies and our results further suggest that IFN-γ and CD43 represent potential targets for immunotherapy in AAA and that selective depletion or blockade of CD43+CD8+ T cell activities merits further investigation, as it may have beneficial effects in other IFN-γ- and CD8+ T cell-dependent diseases.

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Disclosures

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