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Priority Report

Inactivation of Junctional Adhesion Molecule-A Enhances Antitumoral Immune Response by Promoting Dendritic Cell and T Lymphocyte Infiltration

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Abstract

Junctional adhesion molecule-A (JAM-A)–null dendritic cells (DCs) are more motile and effective than their wild-type counterpart in promoting contact hypersensitivity reaction. Here, we show that the growth and aggressiveness of pancreatic islet cell carcinoma induced by SV40 T antigen expression in β cells (Rip1Tag2 mice) are significantly reduced in JAM-A–null mice. Because these tumor cells do not express JAM-A, we focused on changes in stroma reactivity. In the absence of JAM-A, tumors showed a small but significant reduction in angiogenesis and a marked increase in the immune reaction with enhanced infiltration of DCs (CD11c^{+} and MHC-II^{+}) and CD4^{+} and CD8^{+} lymphocytes. In contrast, phagocyte number was not affected. DC capacity to produce cytokines was not significantly altered, but transmigration through JAM-A–null endothelial cells was increased as compared with JAM-A–positive endothelium. On adoptive transfer, JAM-A^{−/−} DCs were recruited to tumors at slightly but significantly higher rate than JAM-A^{+/+} DCs. Ablation of CD4^{+} and CD8^{+} cells with specific antibodies abrogated the inhibitory effect of JAM-A deletion on tumor growth and angiogenesis. These findings support the idea that, in the Rip1Tag2 tumor model, abrogation of JAM-A reduces cancer development by increasing antitumor immune response. *Cancer Res; 70(5); 1759–65. ©2010 AACR.*

Introduction

Junction adhesion molecule-A (JAM-A) is a transmembrane small immunoglobulin-like molecule expressed at endothelial and epithelial tight junctions (1–4). JAM-A is also present on leukocytes where it promotes cell adhesion and motility (1–5). Genetic or antibody-mediated inactivation of JAM-A in mice reduced neutrophil or monocyte infiltration in several models of inflammation such as meningitis, peritonitis, and heart and liver ischemia-reperfusion injury (1–5). In contrast to phagocytes, JAM-A–null dendritic cells (DCs) exhibit increased motility on extracellular matrix proteins and through lymphatic endothelium (6). Consistently, in JAM-A–null mice, DCs move more effectively to lymph nodes and promote enhanced contact hypersensitivity reaction (6). Although the reason for the discrepant behavior of JAM-A in dendritic and inflammatory cells is not fully clear yet, a likely explanation is that DC migration within a three-dimensional tissue stroma follows a different molecular mechanism than that of neutrophils or monocytes (7).

DCs work as antigen-presenting cells promoting T lymphocyte clonal expansion and initiating the adaptive immune response (8). These cells, therefore, may also promote host immunologic reaction against tumors. We crossed Rip1Tag2 mice, which express SV40 T antigen under the control of the rat insulin promoter, with JAM-A^{−/−} mice. Rip1Tag2 mice develop pancreatic islet hyperplasia and highly vascularized adenomas, which progress to invasive carcinomas (9). Importantly, tumor cells in Rip1Tag2 mice do not express JAM-A at any stage of progression, allowing the study of the specific role of JAM-A in the cells of the tumor stroma.

The present study shows that Rip1Tag2 tumors grow less in JAM-A^{−/−} mice, and this effect is accompanied by enhanced infiltration of DCs and CD8^{+} and CD4^{+} T cells and reduced angiogenesis. T-cell infiltration is required for the inhibition of tumor growth in JAM-A^{−/−} mice, implying that the loss of JAM-A promotes a stronger immunologic response against the tumor.

Materials and Methods

Animals. Rip1Tag2 transgenic mice were generated by D. Hanahan (University of California, San Francisco, San Francisco, CA; ref. 9) and provided by G. Christofori (University of Basel, Basel, Switzerland). They were crossed with JAM-A^{−/−} mice (6) to obtain a Rip1Tag2;JAM-A^{−/−}
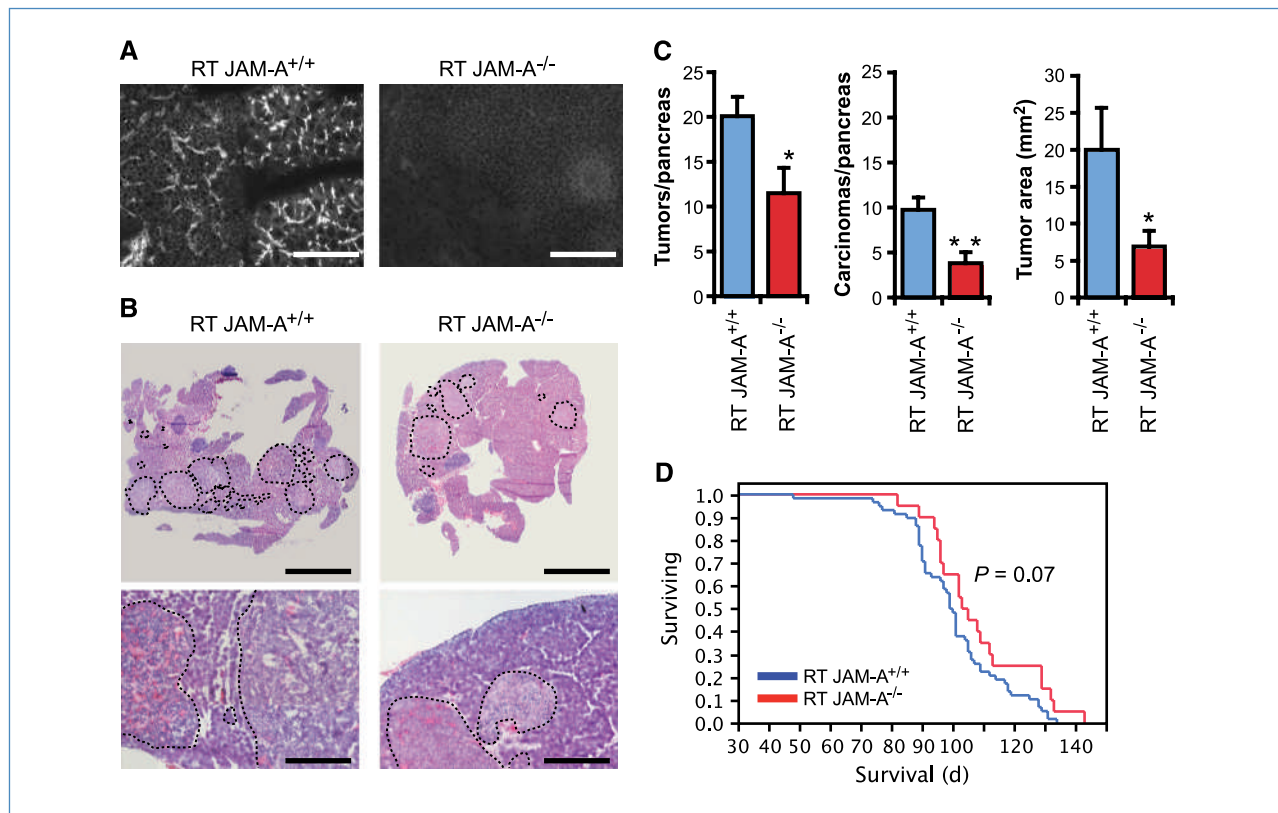
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Figure 1. Rip1Tag2(RT);JAM-A$^{-/-}$ mice show decreased islet tumor growth. A, JAM-A staining of RT JAM-A$^{+/+}$ and RT JAM-A$^{-/-}$ mice. Bar, 100 μm. B, representative H&E staining of pancreas specimens of 13-wk-old mice. The dotted line encircles islet tumors. Bar, 4 mm (top) and 100 μm (bottom). C, quantification of tumor and carcinoma number and cumulative area of islet tumors. Columns, mean of 13 RT JAM-A$^{+/+}$ and 11 RT JAM-A$^{-/-}$ mice; bars, SEM. D, life span of RT JAM-A$^{+/+}$ and RT JAM-A$^{-/-}$ (n = 58 and 20, respectively).

strain. Rip1Tag2;JAM-A$^{+/+}$ littermates, obtained during breeding of the two strains, were used as control.

Histopathologic analysis. Histologic analysis was done as described (10). The following antibodies were used: CD4 (L3T4), CD8a (Ly2), CD11b (Mac-1), CD11c (N418), and MHC class II (MHC-II) were purchased from BD Biosciences. CD31 [platelet endothelial cell adhesion molecule-1 (PE-CAM1)], CD68, F4/80, and Ki-67 were obtained from Chemicon, Hycult Biotechnology, Serotec, and Abcam, respectively. TdT-mediated dUTP nick end labeling (TUNEL) staining was done using the In Situ Cell Death Detection Kit (Roche).

Assessment of tumor burden and tumor number. The length and width of each pancreas tumor were measured microscopically. The formula 0.5 width × 0.5 length × π for approximating the area was applied.

Flow cytometry analysis. Pancreatic islet tumor and peripancreatic lymph nodes were isolated under a stereomicroscope. Single-cell suspensions were stained and analyzed with a FACSCalibur (CellQuest software, BD Biosciences).

DC adoptive transfer. Murine DCs were generated from bone marrow of JAM-A$^{+/+}$ and JAM-A$^{-/-}$ mice as previously described (11). DCs were then incubated with carboxyl-fluorescein-succinimidyl ester (CFSE; Molecular probes) or Far Red DDAO-SE (Molecular probes) for labeling. Green CFSE–labeled JAM-A$^{+/+}$ DCs and Far Red DDAO-SE–labeled JAM-A$^{-/-}$ DCs (1×10^7) were injected i.p. in Rip1Tag2 mice. Mice were sacrificed 48 h after injection, and pancreas specimens were collected for analysis of cell infiltration.

Transmigration assay. JAM-A–null endothelial cells or JAM-A–null endothelial cells reconstituted with full-length JAM-A cDNA (12) were seeded on 5-μm-pore-size filters of 24-well Transwell plates (Costar). Purified DCs were seeded on endothelial monolayers and cell migration was evaluated as described previously (6, 11).

Antibody treatment. Antibodies (50 μg) neutralizing CD4 (clone GK1.5) and CD8 (clone 2.43) and rat IgG isotype control (Sigma) were injected i.p. twice a week in Rip1Tag2;JAM-A$^{+/+}$ or Rip1Tag2;JAM-A$^{-/-}$ mice between the age of 4 and 10 wk.

Statistical analysis. Statistical significance was evaluated by paired or unpaired Student's t test. Significance was defined at $P < 0.05$ (*) and $P < 0.01$ (**). Statistical calculations and Kaplan-Meier's curves were done with JMP 5.1 software (SAS Cary).

Results

Rip1Tag2;JAM-A$^{-/-}$ mice show decreased tumor progression and aggressiveness. By immunofluorescence staining, JAM-A was shown in epithelia of normal pancreatic exocrine

ducts and endothelia of tumor-associated vessels in Rip1-Tag2;JAM-A$^{+/+}$ mice, whereas it was absent in tumor cells. Several cell types infiltrating the tumor stroma were positive for JAM-A expression, such as DCs, monocyte/macrophages, and CD4 and CD8 lymphocytes (Supplementary Fig. S1). As expected, JAM-A was undetectable in any cell type of Rip1Tag2;JAM-A$^{-/-}$ mice (Fig. 1A; Supplementary Fig. S1). Thirteen-week-old Rip1Tag2;JAM-A$^{+/+}$ mice developed around 20 islet tumors per mouse, about 50% of which showed carcinoma features (Fig. 1B). Rip1Tag2;JAM-A$^{-/-}$ mice exhibited lower tumor incidence, smaller tumor size, and lower rate of progression to invasive carcinoma (Fig. 1B and C). In the absence of JAM-A, a small increase in mouse survival was also observed (Fig. 1D) although the difference did not reach statistical significance ($P = 0.07$). This weak difference may be due to the fact that Rip1Tag2 mice die not only of the tumor burden but also of high insulin levels (9). Abrogation of JAM-A expression caused a significant decrease in vascular density (Fig. 2A) and an increase in tumor cell apoptosis, whereas tumor cell proliferation was unaffected (Fig. 2C and B, respectively). Endothelial cell apoptosis, growth, and permeability to dextran (Supplementary Figs. S2 and S3) were unaffected by the lack of JAM-A.

The loss of JAM-A enhances tumor infiltration by DCs and CD4$^+$ and CD8$^+$ T lymphocytes. Tumor infiltration of DCs (MHC-II$^+$ and CD11c$^+$) was higher in Rip1Tag2;JAM-A$^{-/-}$

than in Rip1Tag2;JAM-A$^{+/+}$ mice. Conversely, no difference was observed in the number of infiltrated phagocytes (F4/80$^+$ and CD11b$^+$; Fig. 3A and B). Consistently, peripancreatic lymph nodes showed higher infiltration of CD11c$^+$ cells, but not CD11b$^+$ cells (Fig. 3B). The transfer of JAM-A$^{+/+}$ or JAM-A$^{-/-}$ bone marrow DCs into 13-week-old Rip1Tag2 mice resulted in a small but significant increase in infiltration of JAM-A$^{-/-}$ compared with that of JAM-A$^{+/+}$ DCs into the islet tumors (Fig. 3C).

JAM-A expression did not influence other DC functions such as cytokine production or induction of Th1 polarization of naive CD4 lymphocytes (Supplementary Fig. S4A and B). Furthermore, *in vitro* JAM-A$^{-/-}$ endothelial cells are more permeable to DCs (Fig. 3D).

The increased infiltration of JAM-A$^{-/-}$ DCs correlated with higher numbers of CD4 and CD8 leukocytes in pancreatic islet tumors of Rip1Tag2;JAM-A$^{-/-}$ mice compared with Rip1Tag2;JAM-A$^{+/+}$ mice (Fig. 4A and B). Antibodies directed to CD4 and CD8 cells, which strongly reduced their number in the circulation (Supplementary Fig. S5), abrogated the inhibitory effect of JAM-A inactivation on tumor incidence and progression, angiogenesis, and tumor cell apoptosis (Fig. 4C and D).

Discussion

In this article, we report that the genetic abrogation of JAM-A expression reduces the formation and progression
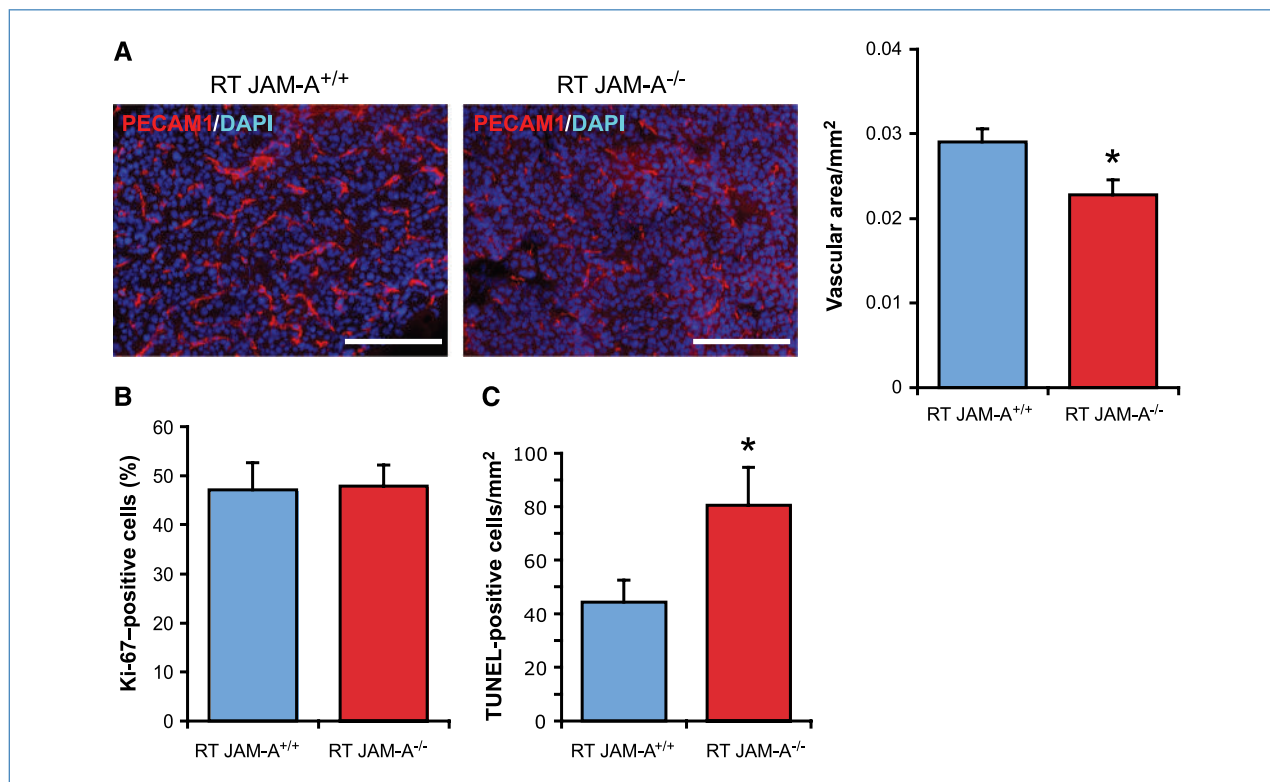

Figure 2. Rip1Tag2(RT);JAM-A$^{-/-}$ mice show decreased angiogenesis and increased apoptosis in pancreatic islet tumors. A, representative PECAM1 staining of blood vessels of pancreatic islet tumors of 13-wk-old RT JAM-A$^{+/+}$ and RT JAM-A$^{-/-}$ mice. Columns, means PECAM1-positive areas per square millimeter ($n = 5$ mice); bars, SEM. Bar, 50 μm. B and C, tumor cell proliferation determined by Ki-67 (B) and apoptosis determined by TUNEL staining (C). Columns, mean positive cells per total tumor cells or per square millimeter, respectively ($n = 5$ mice); bars, SEM.
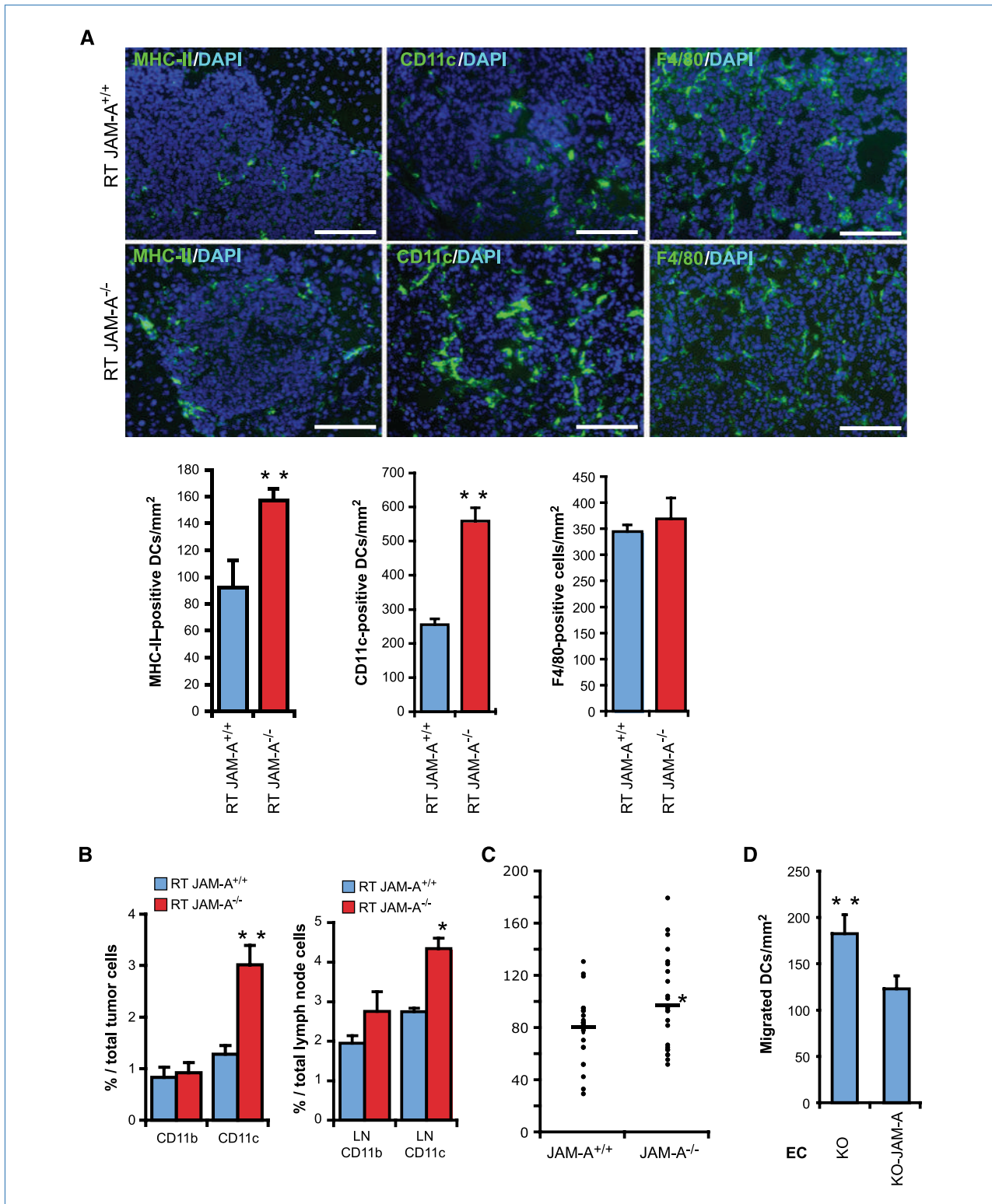
Figure 3. JAM-A–null DCs infiltrate pancreatic islet tumors more efficiently than wild-type cells. A, representative pictures and quantification of MHC-class II^{+} and CD11c^{+} DCs and F4/80^{+} phagocytes in pancreatic islet tumors of RT JAM-A^{+/+} and RT JAM-A^{−/−} mice. Bar, 100 μm. B, flow cytometry profiles of CD11b^{+} and CD11c^{+} cells in total cell suspensions of islet tumors and peripancreatic lymph nodes. C, quantification of adoptively transferred JAM-A^{+/+} and JAM-A^{−/−} DCs into pancreatic islet tumors. D, transmigration of DCs through JAM-A^{+/+} and JAM-A^{−/−} endothelial monolayers. Columns, mean ($n = 5$); bars, SEM.
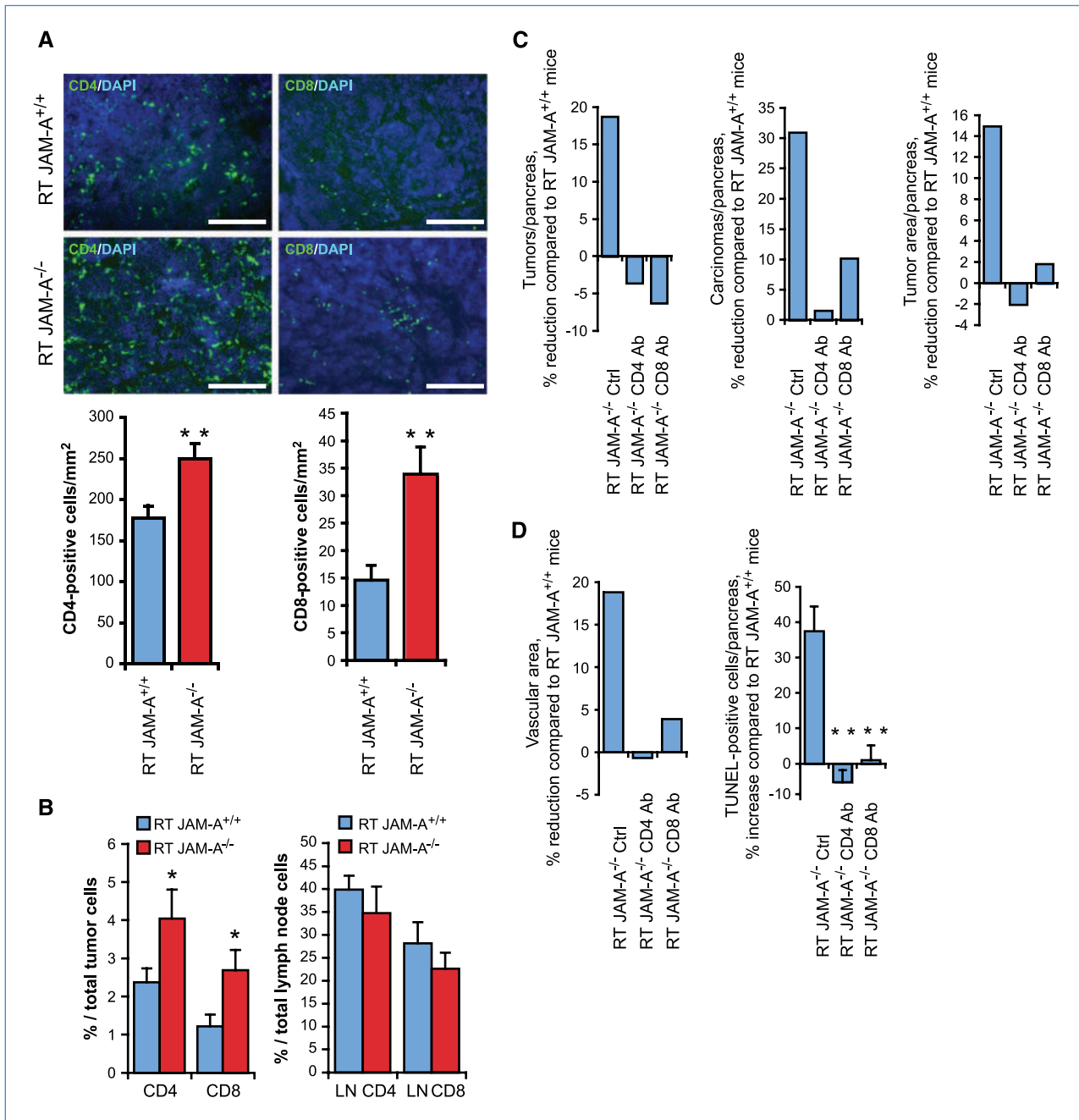
Figure 4. Higher infiltration of CD4$^+$ and CD8$^+$ leukocytes in Rip1Tag2(RT);JAM-A$^{-/-}$ mice. A, representative pictures and quantification of CD4$^+$ and CD8$^+$ leukocytes in pancreatic islet tumors of RT JAM-A$^{+/+}$ and RT JAM-A$^{-/-}$ mice. Columns, mean [n = 5 (20 regions per mouse)]; bars, SEM. Bar, 100 μm. B, flow cytometry profiles of CD4$^+$ and CD8$^+$ cells in total cell suspensions of tumors and peripancreatic lymph nodes. Columns, mean (n = 5); bars, SEM. C, percent reduction of tumor number and area in RT JAM-A$^{-/-}$ compared with RT JAM-A$^{+/+}$ mice on treatment with control or CD4 and CD8 antibodies. D, percent reduction of tumor angiogenesis and percent increase in tumor cell apoptosis by TUNEL staining of tumor sections of RT JAM-A$^{-/-}$ compared with RT JAM-A$^{+/+}$ on treatment with control or CD4 and CD8 antibodies. Columns, mean (n = 8 mice per group); bars, SEM.

of β-cell tumors in Rip1Tag2 mice. In this model, tumor cells do not express JAM-A, and therefore, the observed effects are likely due to functional changes of the cells of the tumor stroma. In the absence of JAM-A, tumor angiogenesis in Rip1Tag2 mice is reduced, supporting previous data on cultured endothelial cells that implicated a role of JAM-A in angiogenesis via α$_v$β$_3$-integrin activation and fibroblast growth factor signaling (1, 13, 14). The decrease in tumor vascularization may be responsible of the increase in tumor cell apoptosis and reduced tumor size. Other groups reported that

matrix metalloproteinase-9 expressed in infiltrating neutrophils mediates the initial angiogenic switch (15). However, neutrophil infiltration is rather low in our experimental conditions and does not change in the absence of JAM-A. In contrast, ablation of lymphocyte CD4 and CD8 (see below) abrogated the inhibition of angiogenesis in JAM-A–null mice, suggesting that the effect may be indirectly mediated by the immune reaction of the stroma.

Our data also suggest that additional mechanisms evoked by JAM-A depletion may contribute to inhibition of tumor growth. In a previously published work, the Rip1Tag2 model was shown to be quite insensitive to self-reactive lymphocytes possibly because of a relatively scarce infiltration of these cells in the tumor stroma and the inhibitory activity of the tumor microenvironment (16). In the present article, we observed a higher rate of DC migration into the tumor, accompanied by increased infiltration of CD4 and CD8 T-lymphocytes. *In vitro* data show that, whereas several DC functions such as production of cytokines or Th1 activation were unchanged in the absence of JAM-A, DC transmigration through JAM-A–null endothelium was increased.

The role of JAM-A in leukocyte transmigration through endothelial cells and in leukocyte motility has been widely investigated (1–6). Data show that the mechanism of action of JAM-A is context and cell type dependent (2, 17). This protein may exert different and, in some cases, even opposite effects in different cell types. In a previously published work, in the effort to investigate the mechanism of action of JAM-A, we found that this molecule may interact with integrins and modulate their internalization and recycling likely through the small GTPase Rap-1 (18). The direct or indirect interactions of JAM-A with different types of integrins and in different cell types have been previously described (14, 18).

The increase in recruitment of CD4^{+} and CD8^{+} lymphocytes in the absence of JAM-A is likely mediated by DCs because lymphocyte infiltration per se in inflammatory tissues is quite insensitive to or even decreased by JAM-A ablation (19). DCs may release activating cytokines and chemokines, which, in turn, attract T lymphocytes. These cells can then mature within the tumor stroma and prime and boost T cells by effective cross-priming of cellular antigens. Consistently, we report here that the decrease in tumor growth is accompanied by increased apoptosis of tumor cells, which may be mediated by cytotoxic CD8 cells. This hypothesis is supported by the results obtained with blocking CD4 and CD8 antibodies, which abrogate the inhibitory effect of JAM-A ablation on tumor growth and apoptosis.

In other systems, JAM-A promotes monocyte recruitment at inflammatory sites (4); however, in JAM-A$^{-/-}$ tumors, we could not detect any significant decrease in phagocyte infiltration in the tumor stroma. Because the effects of JAM-A are dependent on the type of model and inflammatory cytokines implicated (17), it is possible that in Rip1Tag2 tumors, the inhibitory role of JAM-A deficiency such as neutrophils and monocytes is overcome by a strong activation of DCs, CD4, and CD8.

Other studies reported that, in mammary carcinoma cell lines, JAM-A expression reduced tumor cell migration and invasion (20). Because breast tumor expressed JAM-A, these events were related to the tumor-associated protein and implicated the JAM-A–dependent regulation of tumor cell-cell and cell-substrate adhesion, whereas the present study describes the effects of JAM-A on tumor-associated immune cells. How these effects of JAM-A may influence the overall mammary tumor growth *in vivo* remains to be evaluated.

Disclosure of Potential Conflicts of Interest

Grant Support

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Cancer Research

Correction: Online Publication Dates for *Cancer Research* April 15, 2010 Articles

The following articles in the April 15, 2010 issue of *Cancer Research* were published with an online publication date of April 6, 2010 listed, but were actually published online on April 13, 2010:

Garmy-Susini B, Avraamides CJ, Schmid MC, Foubert P, Ellies LG, Barnes L, Feral C, Papayannopoulou T, Lowy A, Blair SL, Cheresh D, Ginsberg M, Varner JA. Integrin α4β1 signaling is required for lymphangiogenesis and tumor metastasis. Cancer Res 2010;70:3042–51. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3761.

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Correction

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