Netrin-1 Signaling Dampens Inflammatory Peritonitis

Valbona Mirakaj, Dimitra Gatidou, Claudia Pöttsch, Klemens König and Peter Rosenberger

J Immunol 2011; 186:549-555; Prepublished online 22 November 2010; doi: 10.4049/jimmunol.1002671
http://www.jimmunol.org/content/186/1/549

Supplementary Material  
http://www.jimmunol.org/content/suppl/2010/11/19/jimmunol.1002671.DC1

References  
This article cites 36 articles, 16 of which you can access for free at:  
http://www.jimmunol.org/content/186/1/549.full#ref-list-1

Subscription  
Information about subscribing to The Journal of Immunology is online at:  
http://jimmunol.org/subscription

Permissions  
Submit copyright permission requests at:  
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts  
Receive free email-alerts when new articles cite this article. Sign up at:  
http://jimmunol.org/alerts
Netrin-1 Signaling Dampens Inflammatory Peritonitis

Valbona Mirakaj,∗† Dimitra Gatidou,∗ Claudia Pötzsch,* Klemens König,† and Peter Rosenberger∗†

Previous studies implicated the anti-inflammatory potential of the adenosine 2B receptor (A2BAR). A2BAR activation is achieved through adenosine, but this is limited by its very short t1/2. To further define alternative adenosine signaling, we examined the role of netrin-1 during acute inflammatory peritonitis. In this article, we report that animals with endogenous repression of netrin-1 (Ntn1−/−) demonstrated increased cell count, increased peritoneal cytokine concentration, and pronounced histological changes compared with controls in a model of zymosan A peritonitis. Exogenous netrin-1 significantly decreased i.p. inflammatory changes. This effect was not present in animals with deletion of A2BAR (A2BAR−/−), A2BAR−/− animals demonstrated no change in cell count, i.p. cytokine concentration, or histology in response to netrin-1 injection. These data strengthen the role of netrin-1 as an immunomodulatory protein exerting its function in dependence of the A2BAR and further define alternative adenosine receptor signaling. The Journal of Immunology, 2011, 186: 549–555.

The activation of immune cells and the subsequent inflammatory response are critical steps during immune surveillance and host defense facilitating the elimination of foreign or infectious agents (1–4). Leukocyte migration and the control of an inflammatory response are tightly controlled multistep processes and have been well characterized in recent years (4, 5). Control mechanisms exist to prevent an exaggerated inflammatory response that might result in exaggerated tissue destruction and subsequent organ dysfunction. Only recently, neuronal-guidance proteins were identified to be part of these control mechanisms and, as such, to attenuate leukocyte infiltration and the associated cytokine release (6–10). The neuronal-guidance protein netrin-1 is involved in the control of local inflammation during tissue hypoxia and the early stages of acute lung injury, and its anti-inflammatory function is dependent on the interaction of netrin-1 with the adenosine 2B receptor (A2BAR) (11–13). This opens the possibility of endogenous adenosine receptor activation independent of the short-lived nucleoside adenosine.

Several reports demonstrated a protective role for adenosine and reported the tissue-protective potential of A2BAR activation. As such, signaling through A2BAR dampens pulmonary inflammation and controls fluid exchange during acute lung injury (14, 15). During ischemia–reperfusion injury, signaling through A2BAR reduces the associated reperfusion injury and possesses tissue-protective potential (16, 17). The coordinated induction of A2BAR during cellular hypoxia and an acute inflammatory process has to be seen as an adaptation of the affected tissues to increase vascular barrier response and to induce tissue-protective signaling through the A2BAR (18–21). However, a major limitation of adenosine signaling is that once adenosine is generated into the extracellular milieu, it is also rapidly cleared through passive or active uptake. This occurs through nucleoside transporters, termed equilibrative nucleoside transporters and concentrative nucleoside transporters, respectively. As a result, adenosine action is marked by its very short t1/2 (22, 23). Systemic administration of adenosine as an anti-inflammatory drug is not possible because this results in a variety of undesirable side effects, such as bradycardia or vasodilation (24). Therefore, mechanisms that are able to signal through the A2BAR are highly desirable and need further definition.

Given the role of netrin-1 during hypoxic inflammation, we aimed to define its role during acute exudative inflammation and corroborate a possible role for the A2BAR. In this article, we report that netrin-1 is expressed in intestinal organs, and this expression is reduced during acute peritoneal inflammation. Animals with endogenous repression of netrin-1 (Ntn1−/−) significantly dampened an increased inflammatory response following zymosan A (ZyA)-induced peritonitis compared with littermate controls. The injection of exogenous netrin-1 significantly dampened the extent of inflammatory peritonitis; this was dependent on the A2BAR. Therefore, these data corroborate the role of netrin-1 as an endogenous anti-inflammatory protein with significant influence on inflammatory control that is dependent on the A2BAR.

Materials and Methods

Transcriptional and protein analysis

Murine transcriptional analysis of Ntn1 mRNA was performed using sense primer 5′-GAG CGG GGG AGT CTG TCT-3′ and antisense primer 5′-TGG TTT GAT TGC AGG TCT CTG T-3′. Murine β-actin expression was evaluated using sense primer 5′-CTC TCC CTC CAC ACA GCA TGG CTG T-3′ and antisense primer 5′-TCA CGC ACC AAT TCC TCA TTG T-3′. Semiquantitative analysis of human NNT1 mRNA expression was performed using primer sets containing sense primer 5′-GAG TGC TTC GTC GCC TGT AAC-3′ and the antisense primer 5′-AGG CAG ACA CCT CCG CTC TT-3′. Samples were controlled for β-actin using the following primers: sense 5′-GGT GCC TTT TAG CAT GTC GAC AAG-3′, antisense 5′-ACT...
GGA ACG GTG AAG GTG ACA G-3’. All PCR analyses were performed using real-time PCR (iCycler; Bio-Rad, Hercules, CA), as described previously (12).

For Western blot analysis, animal samples were homogenized, normalized for protein levels, and applied in nonreducing conditions to SDS containing polyacrylamide gels. Abs used for Western blotting included rabbit polyclonal anti–netrin-1 (sc 20786; Santa Cruz Biotechnology, Santa Cruz, CA) for murine Ntn1 analysis. Actin was stained using rabbit anti-Actin (Cell Signaling Technology, Beverly, MA). Blots were washed, and species-matched peroxidase-conjugated secondary Ab (Santa Cruz Biotechnology) was added. Labeled bands from washed blots were detected by ECL (Amersham Biosciences, Piscataway, NJ).

**Immunofluorescent staining**

For murine immunofluorescence, animals were sacrificed; colon and peritoneum were embedded in paraffin; and tissue sections were placed on slides, air-dried, and fixed in methanol followed by 4% acetone. Air-dried tissue sections were washed three times in PBS and blocked with 5% BSA (Sigma-Aldrich, St. Louis, MO). Rabbit polyclonal anti–netrin-1 (sc 20786; Santa Cruz Biotechnology), at a dilution of 1:1000, was used as the primary Ab. Texas Red 545-conjugated goat anti-rabbit (Invitrogen, Eugene, OR) was used as the secondary Ab. DAPI (Invitrogen) was used for nuclear counterstaining.

**Histopathological evaluation**

Following peritonitis, mice were killed, and tissues were fixed in 10% formaldehyde solution. Tissues were embedded in paraffin and stained with H&E. Fluorescence microscope LSM 510 Meta (Carl Zeiss, Jena, Germany) was used for imaging.

**Peritonitis model**

All animal experiments were in accordance with the regulations of the Regierungspräsidium Tübingen and the local ethical committee. Mice were injected i.p. with 1 ml ZyA (1 mg/ml; Sigma-Aldrich), and subsequently with vehicle (saline + 0.2% BSA) or 1 μg recombinant murine netrin-1 (R&D Systems, Minneapolis, MN) in 150 μl total volume. Recruited leukocytes were obtained at the described time points by peritoneal lavage with calcium- and magnesium-free ice-cold PBS solution containing 10 U/ml unfractionated heparin. Collected cells were washed, resuspended in 2 ml HBSS, and counted, and cytospin samples were prepared. All reagents used were endotoxin-free.

**Wild-type, Ntn1+/−, and A2BAR−/− animals**

Approval of the Institutional Review Board and the Regierungspräsidium Tübingen was obtained. Wild-type (WT), Ntn1+/−, and A2BAR−/− mice and littermate control mice were bred and genotyped, as described previously (13).

**Measurement of serum cytokines**

TNF, IL-1β, IL-6, and IL-8 were measured in the peritoneal lavage of WT, Ntn1+/−, and A2BAR−/− animals by standard ELISA (R&D Systems).

**Measurement of peritoneal cAMP**

After peritoneal lavage was obtained, cAMP concentrations were determined with a cAMP EIA Kit (Cayman Chemical, Ann Arbor, MI).

**Transcriptional analysis of TNF-α and IL-6**

Human CaCo-2 cells were incubated with ZyA (100 μg/ml) for 12 h to determine transcriptional response of TNF-α and IL-6. Netrin-1 (1 μg/ml) was added, or cells were preincubated with the A2BAR antagonist 1-propyl-8-(p-sulfophenyl)xanthine (10 μM; Sigma-Aldrich) for 30 min prior to netrin-1 addition. Transcriptional analysis of TNF-α was performed using primer sense 5′-TGG TGG TGG TGG TGG TGG GCT ACA GGC TTC ACA ACC CTA CTA CTT TGT TTG CTC ACA GTC AAT GG-3′ and antisense 5′-GGG GGC CAG CAA CTC AGA AAA CT-3′; IL-6 primer used was sense 5′-AGC GCC TTC GGT CCA GTT GC-3′ and antisense: 5′-GGT GCT GGT GTC TGT GGG CGG CG-3′.

**Transcriptional analysis of netrin-1R expression**

Murine tissues were obtained from the liver and the intestine to determine the expression levels of A2BAR using sense: 5′-GGA AGG ACT TCG TCT CTC CA-3′ antisense: 5′-GGG GGC CAG CAA CTC AGA AAA CT-3′, neogenin sense: 5′-GCA AAA CTC GCG ACC CCC AAT-3′ antisense: 5′-GCT GCT CTC ACA GTC AAT GG-3′, and UNCSb sense: 5′-GGA CCT CCT CAG TGC TAC A-3′ antisense: 5′-GCT AAG TCG TCC TGC CAC ATC CT-3′ primer in mice.

**Data analysis**

All data are presented as mean ± SEM from 9–11 animals per condition. GraphPad 5.0 software (GraphPad, San Diego, CA) was used for statistical analysis. We performed statistical analysis using one-way ANOVA to determine group differences using post hoc analysis, followed by the unpaired Student t test (two sided, α < 0.05). A p value <0.05 was considered statistically significant.

**Results**

Netrin-1 is repressed during an acute inflammatory response

We initially addressed the question of whether netrin-1 is expressed outside the CNS in intestinal organs to validate the model subsequently used; we found a robust expression of netrin-1 within several murine organs (Fig. 1A, 1B). We then aimed to identify whether netrin-1 expression is affected during an acute inflammatory process.
in the peritoneal cavity in vivo and found a significant reduction in netrin-1 in response to sterile peritonitis induced through ZyA (Fig. 1C, 1D). To corroborate this finding, we tested whether netrin-1 expression was changed following inflammatory stimulation in vitro and exposed the intestinal CaCo-2 cell line to TNF-α and IL-6 stimulation. This corroborated that netrin-1 expression is significantly reduced in intestinal epithelial cells in response to inflammatory cytokines (Fig. 1E, 1F).

Endogenous netrin-1 controls acute inflammatory peritonitis

We then proceeded to investigate the functional role of endogenous netrin-1 repression and its role during acute inflammatory peritonitis. For this purpose, we exposed previously characterized Ntn1+/− mice (Ntn1−/− are not viable) and initially determined the expressional levels of netrin-1 receptors in these animals but did not find a significant difference between Ntn1+/+ and Ntn1+−/− animals (Supplemental Fig. 1). Following i.p. ZyA injection, we found that Ntn1+−/− mice exhibited significantly increased cell numbers in the peritoneal lavage compared with Ntn1+/+ animals (3 × 10⁶, 4.2 ± 0.29 versus 2.9 ± 0.39, respectively; p < 0.01) (Fig. 2A). This result was reflected when evaluating the myeloperoxidase (MPO) activity within the peritoneal fluid and the protein content within the peritoneal exudate (Fig. 2B, 2C). We also performed histological evaluation of the peritoneal cavity, the mesenterial fat, and the cellular exudate. We found that Ntn1+/− mice exhibited significantly pronounced inflammatory changes and tissue destruction of the peritoneal tissue and increased infiltration of inflammatory cells into the mesenterial fat tissue compared with Ntn1+/+ animals (Fig. 2D). Cytospin samples verified the increased cell count in the peritoneal lavage of Ntn1+/− mice following ZyA-induced peritonitis compared with Ntn1+/+ animals. When evaluating inflammatory cytokines within the peritoneal lavage, Ntn1+/− mice exhibited significantly increased levels (pg/ml) of TNF-α, IL-1β, IL-6, and IL-8 compared with Ntn1+/+ animals (67 ± 10 versus 38 ± 6, p < 0.01; 1322 ± 445 versus 762 ± 78, p < 0.05; 2479 ± 696 versus 1369 ± 270, p < 0.05; and 4993 ± 836 versus 3268 ± 648, p < 0.05) (Fig. 3). Intraperitoneal levels of cAMP were also significantly different between Ntn1+/+ and Ntn1+/− animals (Supplemental Fig. 2).

Exogenous netrin-1 dampens inflammatory peritonitis

We next investigated the functional impact of exogenous netrin-1. We used WT animals and exposed them to ZyA peritonitis, with subsequent injection of vehicle or netrin-1. In the animals injected with netrin-1, we found a significantly reduced cell count (3 × 10⁶) in the peritoneal lavage compared with vehicle-injected animals (NaCl: 0.59 ± 0.07; 2.24 ± 0.15 versus 3.83 ± 0.25, respectively, p < 0.01) (Fig. 4A). This result was corroborated by the measurement of MPO activity within the peritoneal fluid and the protein content in the peritoneal lavage (Fig. 4B, 4C). In the histological sections of these animals, the peritoneal tissue and the mesenterial fat of animals injected with netrin-1 demonstrated reduced signs of inflammation compared with vehicle controls (Fig. 4D). Cytospin samples of the peritoneal lavage confirmed this finding demonstrating reduced cell count in the netrin-1–treated animals compared with vehicle controls. We then

![FIGURE 2. Endogenous netrin-1 dampens inflammatory peritonitis. Ntn1+/+ and Ntn1−/− animals were injected i.p. with NaCl 0.9% or 1 mg of ZyA; samples were taken after 4 h. Cell count (A), MPO activity (B), protein content (C), and representative histological analysis of the peritoneum, the mesenterial fat, and cytospin samples (D) in the peritoneal lavage. Sections were stained with H&E (original magnification ×400; insets ×1000). Data are mean ± SEM (n = 11 per group). *p < 0.05; **p < 0.01; ***p < 0.001.](http://www.jimmunol.org/Downloaded from http://www.jimmunol.org/)

The Journal of Immunology 551
evaluated inflammatory cytokines within the peritoneal lavage and found that exogenous netrin-1 significantly reduced the concentration (pg/ml) of TNF-α (Fig. 5A), IL-1β (Fig. 5B), IL-6 (Fig. 5C), and IL-8 (Fig. 5D) compared with vehicle treatment (NaCl: 3 ± 2, 15.5 ± 4 versus 32 ± 5, p < 0.05; NaCl: 37 ± 23, 435 ± 56 versus 1897 ± 650, p < 0.05; NaCl: 6 ± 2, 860 ± 318 versus 2965 ± 617, p < 0.05; and NaCl: 16 ± 5, 1467 ± 476 versus 3823 ± 930, p < 0.05, respectively). Intraperitoneal levels of cAMP were significantly reduced in the ZyA+Netrin-1 group compared with the ZyA+Vehicle group (Supplemental Fig. 3).

Exogenous netrin-1 does not reduce inflammatory peritonitis in A2BAR−/− animals

First, we determined the expressional levels of potential netrin-1Rs in A2BAR−/− animals to exclude the influence of A2BAR deletion. We found a significant difference only for A2BAR expression in these animals, not for other potential netrin-1Rs (Supplemental Fig. 4). To evaluate whether the effect of netrin-1 during an exudative inflammatory process is dependent on A2BAR, we induced ZyA peritonitis in A2BAR−/− animals and injected them with vehicle or netrin-1. There was no change in the cell count (× 10⁶) within the peritoneal lavage of animals injected with netrin-1 compared with vehicle-treated animals (NaCl: 0.25 ± 0.02; 3.83 ± 0.48 versus 3.68 ± 0.39, respectively) (Fig. 6A). There also was no reduction in MPO activity within the peritoneal cavity following injection of netrin-1 in A2BAR−/− animals (Fig. 6B, 6C). Histological sections did not exhibit a reduction in inflammation within the peritoneal cavity or the

FIGURE 3. Endogenous netrin-1 dampens inflammatory cytokine release in vivo. Ntn1+/+ and Ntn1+/− animals were injected i.p. with NaCl 0.9% or 1 mg of ZyA; samples were taken after 4 h. TNF-α (A), IL-1β (B), IL-6 (C), and IL-8 (D) concentration in peritoneal lavage of Ntn1+/+ and Ntn1+/− animals. Data are mean ± SEM (n = 11 per group). *p > 0.05; **p < 0.01; ***p < 0.001.

FIGURE 4. Exogenous netrin-1 dampens inflammatory peritonitis in vivo. WT animals were injected i.p. with NaCl 0.9% or 1 mg of ZyA, followed by i.v. injection with vehicle or recombinant netrin-1 (1 μg); samples were taken after 4 h. Cell count (A), MPO activity (B), protein content (C), and representative histological analysis of the peritoneum, the mesenterial fat tissue, and cytospin samples (D) in the peritoneal lavage. Sections were stained with H&E (original magnification ×400; insets ×1000). Data are mean ± SEM (n = 9 per group). *p < 0.05; **p < 0.01; ***p < 0.001.
mesenterial fat following netrin-1 injection in A2BAR$^{2/2}$ animals (Fig. 6D). Cytospin samples of the peritoneal lavage confirmed this by demonstrating no change in cell count in the netrin-1-injected animals compared with vehicle controls. Finally, when evaluating the concentration of inflammatory cytokines within the peritoneal lavage, we did not find a change in cytokine concentration following exogenous netrin-1 administration in the A2BAR$^{2/2}$ animals. The concentration (pg/ml) of TNF-α (Fig. 7A), IL-1β (Fig. 7B), IL-6 (Fig. 7C), or IL-8 (Fig. 7D) did change following netrin-1 injection compared with vehicle treatment (NaCl: 2.5 ± 0.5, 38 ± 5 versus 38 ± 11; NaCl: 12 ± 4, 545 ± 115 versus 458 ± 90; NaCl: 4 ± 1, 4712 ± 1126 versus 4696 ± 1253; and NaCl: 16 ± 3, 6475 ± 1361 versus 5886 ± 1409, respectively. Intraperitoneal levels of cAMP reflected these results (Supplemental Fig. 5).

To gain additional data about the anti-inflammatory role of netrin-1, we stimulated CaCo-2 cells with ZyA, which resulted in a significant induction of TNF-α and IL-6 transcripts within these cells. When adding netrin-1 to this, we found a significant reduction in the transcriptional response to ZyA. Following pre-incubation of CaCo-2 cells with the A2BAR antagonist 1-propyl-8-(p-sulfophenyl)xanthine, this effect was significantly attenuated but not completely abolished (Supplemental Fig. 6).

**Discussion**

Signaling through the A2BAR possesses anti-inflammatory potential and dampens inflammatory tissue damage. Endogenous activation of A2BAR occurs through adenosine, but recent reports...
suggested a role for the neuronal repellent netrin-1 in A2BAR activation. Therefore, we assessed the role of exogenous and endogenous netrin-1 in a model of inflammatory peritonitis to further define its anti-inflammatory role, as well as its potential to exert its function in dependence of the A2BAR. We report in this article that netrin-1 possesses significant anti-inflammatory potential during exudative inflammation, and this effect is dependent on the A2BAR.

The role of netrin-1 was initially described in the context of axonal growth and migration. Floor plate cells at the ventral midline of the mammalian embryonic neural tube secrete netrin-1 and generate a circumferential gradient of netrin-1 protein in the neuroepithelium (25). This protein gradient possesses bifunctional potential, in that it attracts some axons to, as well as repels other axons from, the ventral midline. Subsequent studies demonstrated the expression of netrin-1 outside the nervous system within the vasculature, a reduction in netrin-1 expression during an acute inflammatory process, and that netrin-1 is a potent inhibitor of myeloid cell migration toward chemotactic stimuli in vitro (12, 26). This anti-inflammatory potential of netrin-1 was corroborated by Wang et al. (27) during renal ischemia–reperfusion injury. In this study, the investigators demonstrated that the administration of netrin-1 resulted in reduced tissue injury and improved kidney function following ischemia–reperfusion injury. Consistent with these findings, we revealed a role for mucosal netrin-1 in the attenuation of hypoxic inflammation (13). Our current findings corroborate the anti-inflammatory potential and the tissue-protective role of netrin-1 during inflammatory peritonitis and indicate a role for A2BAR in mediating netrin-1 action during an acute inflammatory response.

Signaling through A2BAR was suggested to possess protective potential in a variety of organ systems and to be important during the orchestration of an acute inflammatory response (17, 28, 29). As such, A2BAR activation is involved in the inflammatory response of mast cells, epithelial cells, smooth muscle cells, and fibroblasts (30, 31). A2BAR also limits an inflammatory response of the endothelium, determines its permeability, and suppresses macrophage activation, thereby preventing tissue injury after episodes of hypoxia and ischemia (32, 33). Thus, A2BAR might be an important pharmacological target in treating conditions associated with barrier dysfunction and acute inflammation. Although investigations of pharmacological approaches for A2BR activation are underway, a possible endogenous activation of A2BAR through netrin-1 has not been well investigated. The present study demonstrated that netrin-1 does not exert anti-inflammatory function in A2BAR-/- animals and, therefore, showed that its function is dependent on A2BAR.

For central nervous function of netrin-1, the deleted in colorectal cancer receptor and the UNC5 homologs UNC5A, -B, -C, and -D were initially reported (34). Corset et al. (11) subsequently demonstrated that netrin-1 also signals through A2BAR during axonal outgrowth. In this study, netrin-1 stimulation resulted in an intracellular cAMP increase that was dependent on A2BAR. However, other reports implicated that the repulsive function of netrin-1 during axonal growth is independent of A2BAR function; rather, it is mediated through the deleted in colon cancer receptor (35). In a study by McKenna et al. (36), the authors demonstrated that activation or overexpression of A2BAR reduced the level of the repellent UNC5A receptor on the surface of neuronal cells. This resulted in a conversion of repulsion to attraction of these cells. Ly et al. (26) demonstrated that the anti-inflammatory function of netrin-1 was dependent on a member of the UNC family, the UNC5b receptor in vitro through chemotactic migration studies, but they did not translate this finding into in vivo evidence. In recent studies, we demonstrated that netrin-1 dampens hypoxic inflammation through A2BAR and corroborated this during acute lung injury (12, 13). In the present study, we investigated the role of netrin-1 during acute exudative inflammation and assessed its role during inflammatory peritonitis. We found that netrin-1 significantly reduced the number of inflammatory cells and dampened the histological changes of an acute inflammatory response in dependence of the A2BAR. Furthermore, netrin-1 significantly reduced the release of inflammatory cytokines; this was not observed in animals with gene-targeted deletion of A2BAR. Given the findings by McKenna et al. (36), a role for A2BAR in the expression of other known netrin-1Rs on immunocompetent cells might be possible and explain our findings; however, further studies are needed. The results that we presented in this article corroborate an important role for A2BAR for the anti-inflammatory potential of netrin-1.

In summary, our results strengthen the role of netrin-1 as an endogenous modulator of an inflammatory response during an acute exudative inflammation. Exogenous netrin-1 exerts significant anti-inflammation that is dependent on the A2BAR. As such, these findings increase our knowledge about a possible role for adenosine receptor activation during netrin-1 anti-inflammation.

Disclosures

The authors have no financial conflicts of interest.

References

1. Delves, P. J., and I. M. Roitt. 2000. The immune system. First of two parts. N. Engl. J. Med. 343: 37–49.
2. Delves, P. J., and I. M. Roitt. 2000. The immune system. Second of two parts. N. Engl. J. Med. 343: 108–117.
3. Springer, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76: 301–314.
4. Baggioili, M. 1998. Chemokines and leukocyte traffic. Nature 392: 565–568.
5. Luster, A. D. 1998. Chemokines—chemotactic cytokines that mediate inflammation. N. Engl. J. Med. 338: 436–445.
6. Wu, J. Y., L. Feng, H. T. Park, N. Havligoula, L. Wen, H. Tang, K. B. Bacon, Jiang Zh, Zhang Xc, and Y. Rao. 2001. The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors. Nature 410: 948–952.
7. Henes, J., M. A. Schmit, J. C. Morote-Garcia, V. Mirakaj, D. Köhler, L. Glover, T. Eldh, U. Walter, J. Karhausen, S. P. Colgan, and P. Rosenberger. 2009.
Inflammation-associated repression of vasodilator-stimulated phosphoprotein (VASP) reduces alveolar-capillary barrier function during acute lung injury. 

FASER J. 23: 4244–2455.

8. Rao, Y., K. Wong, M. Ward, C. Jurgensen, and J. Y. Wu. 2002. Neuronal migration and molecular conservation with leukocyte chemotaxis. Genes Dev. 16: 2973–2984.

9. Suzuki, K., T. Okuno, M. Yamamoto, R. J. Pasterkamp, N. Takegahara, H. Takamatsu, T. Kitao, J. Takagi, P. D. Rennert, A. L. Kolodkin, et al. 2007. Semaphorin 7A initiates T-cell-mediated inflammatory responses through alpha1beta1 integrin. Nature 446: 680–684.

10. Kruger, R. P., J. Aurandt, and K. L. Guan. 2005. Semaphorin command cells to move. Nat. Res. Mol. Cell Biol. 6: 789–800.

11. Corset, V., K. T. Nguyen-Ba-Charvet, C. Forcet, E. Moyaie, A. Chédotal, and P. Mehlen. 2000. Netrin-1-mediated axon outgrowth and cAMP production interacts with adenosine A2B receptor. Nature 407: 747–750.

12. Mirakaj, V., C. A. Thix, S. Laucher, C. Mielke, J. C. Morote-Garcia, M. A. Schmit, J. Henes, K. E. Unertl, D. Köhler, and P. Rosenberger. 2010. Netrin-1 dampens pulmonary inflammation during acute lung injury. Am. J. Respir. Crit. Care Med. 181: 815–824.

13. Rosenberger, P., J. M. Schwab, V. Mirakaj, E. Masekowsky, A. Mager, J. C. Morote-Garcia, K. Unertl, and H. K. Eltzschig. 2009. Hypoxia-inducible factor-dependent induction of netrin-1 dampens inflammation caused by hypoxia. Nat. Immunol. 10: 195–202.

14. Zhou, Y., A. Mohsenin, E. Morschl, H. W. Young, J. G. Molina, W. Ma, C. X. Sun, H. Martinez-Valdez, and M. R. Blackburn. 2009. Enhanced airway inflammation and remodeling in adenine deaminase-deficient mice lacking the A2B adenosine receptor. J. Immunol. 182: 8037–8046.

15. Eckle, T., A. Grenz, S. Laucher, and H. K. Eltzschig. 2008. A2B adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice. J. Clin. Invest. 118: 3301–3315.

16. Hart, M. L., B. Jacoby, J. Schittenhelm, M. Henn, and H. K. Eltzschig. 2009. Cutting Edge: A2B Adenosine receptor signaling provides potent protection during intestinal ischemia-reperfusion injury. J. Immunol. 182: 3963–3968.

17. Yang, D., Y. Zhang, H. G. Nguyen, M. Koupena, A. K. Chauhan, M. Makitalo, M. R. Jones, C. S. Hilaire, D. C. Seldin, P. Toselli, et al. 2006. The A2B adenosine receptor protects against inflammation and excessive vascular adhesion. J. Clin. Invest. 116: 1913–1923.

18. Eltzschig, H. K., J. C. Ibla, G. T. Furuta, M. O. Leonard, K. A. Jacobson, K. Enjyoji, S. C. Robson, and S. P. Colgan. 2003. Coordinated adenine nucleotide phosphohydrolasis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidasases and adenosine A2B receptors. J. Exp. Med. 198: 783–796.

19. Haskó, G., B. Csóka, Z. H. Németh, E. S. Vizi, and P. Pacher. 2009. A(2B) adenosine receptors in immunity and inflammation. Trends Immunol. 30: 263–270.

20. Kong, T., K. A. Westerman, M. Faigle, H. K. Eltzschig, and S. P. Colgan. 2006. HIF-dependent induction of adenosine A2B receptor in hypoxia. FASEB J. 20: 2242–2250.

21. Kolachala, V. L., M. Vijay-Kumar, G. Dalmasso, D. Yang, J. Linden, L. Wang, A. Gewirtz, K. Ravid, D. Merlin, and S. V. Sitaraman. 2008. A2B adenosine receptor gene deletion attenuates murine colitis. Gastroenterology 135: 861–870.

22. Baldwin, S. A., P. R. Beal, S. Y. Yao, A. E. King, C. E. Cuss, and J. D. Young. 2004. The equilibrative nucleoside transporter family. SLCA2 Pfugers Arch. 447: 753–743.

23. Eltzschig, H. K., P. Abdulla, E. Hoffman, K. E. Hamilton, D. Daniels, C. Schönfeld, M. Löffler, G. Reyes, M. Duszenko, J. Karhausen, et al. 2005. HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. J. Exp. Med. 202: 1493–1506.

24. Belardelli, L., J. C. Shroyock, Y. Song, D. Wang, and M. Srinivas. 1995. Ionic basis of the electrophysiological actions of adenosine on cardiomyocytes. FASEB J. 9: 359–365.

25. Moore, S. W., M. Tessier-Lavigne, and T. E. Kennedy. 2007. Netrins and their receptors. Adv. Exp. Med. Biol. 621: 17–31.

26. Ly, N. P., K. Komatsuzaki, I. P. Fraser, A. A. Tseng, P. Prodhon, K. J. Moore, and T. B. Kinane. 2005. Netrin-1 inhibits leukocyte migration in vitro and in vivo. Proc. Natl. Acad. Sci. USA 102: 14729–14734.

27. Wang, W., W. B. Reeves, and G. Ramesh. 2008. Netrin-1 and kidney injury. J. Renal. Physiol. 294: F739–747.

28. Wang, L., V. Kolachala, B. Walla, S. Baliusbramaniam, R. A. Hall, D. Merlin, and S. V. Sitaraman. 2004. Agonist-induced polarized trafficking and surface expression of the adenosine 2b receptor in intestinal epithelial cells: role of SNARE proteins. Am. J. Physiol. Gastrointest. Liver Physiol. 287: G1100–G1107.

29. Walla, B., F. E. Castaneda, L. Wang, V. L. Kolachala, R. Bajaj, J. Roman, D. Merlin, A. T. Gewirtz, and S. V. Sitaraman. 2004. Polarized fibronectin secretion induced by adenosine regulates bacterial-epithelial interaction in human intestinal epithelial cells. Biochem. J. 382: 589–596.

30. Ryzhov, S., A. E. Goldstein, I. Biaggioni, and I. Feoktistov. 2006. Cross-talk between G(s)- and G(q)-coupled pathways in regulation of interleukin-4 by A(2B) adenosine receptors in human mast cells. Mol. Pharmacol. 70: 727–735.

31. Ryzhov, S., R. Zaynagzidanov, A. E. Goldstein, S. V. Novitskiy, M. S. Bychkov, I. Biaggioni, and I. Feoktistov. 2008. Effect of A2B adenosine receptor gene ablation on adenosine-dependent regulation of proinflammatory cytokines. J. Pharmacol. Exp. Ther. 324: 694–700.

32. Schingnitz, U., K. Hartmann, C. P. Macmanus, T. Ecke, S. Zug, S. P. Colgan, and H. K. Eltzschig. 2008. A2B adenosine receptor dampens endotoxin-induced acute lung injury. J. Immunol. 184: 5271–5279.

33. Eckle, T., M. Faigle, A. Grenz, S. Laucher, L. F. Thompson, and H. K. Eltzschig. 2010. Signaling through the A2B adenosine receptor dampens hypoxia-induced vascular leak. Blood 116: 2024–2035.

34. Rahman, K. 2007. Effects of garlic on platelet biochemistry and physiology. Mol. Nutr. Food Res. 51: 1335–1344.

35. Stein, E., Y. Zou, M. Poo, and M. Tessier-Lavigne. 2001. Binding of DCC by netrin-1 to mediate axon guidance independent of adenosine A2B receptor activation. Science 291: 1976–1982.

36. McKenna, W. L., C. Wong-Staal, G. C. Kim, H. Macias, L. Hinck, and J. L. Bartoe. 2008. Netrin-1 independent adenosine A2b receptor activation regulates the response of axons to netrin-1 by controlling cell surface levels of UNC5A receptors. J. Neurochem. 104: 1081–1090.