Efficient dendritic cell priming of T lymphocytes depends on the extracellular matrix protein mindin

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Rho guanosine triphosphatases (GTPases) regulate multiple aspects of dendritic cell (DC) function, but what regulates the expression of Rho GTPases in DCs is unknown. Here, we show that the extracellular matrix protein mindin regulates the expression of Rho GTPases in DCs. Mindin^{-/-} mice displayed defective CD4⁺ T-cell priming and impaired humoral immune responses to T-dependent antigens. Mindin^{-/-} DCs had reduced expression of Rac1/2 and impaired priming capacity owing to inefficient engagement with T lymphocytes. Ectopic Rac1 expression restored the priming capability of Mindin^{-/-} DCs. Furthermore, we show that DC adhesion to mindin matrix was blocked by antibodies to α_4 , α_5 and β_1 integrins. DCs lacking β_1 integrin had reduced adhesion to mindin matrix, decreased expression of Rac1/2 and impaired priming capacity. These results suggest that mindin–integrin interactions play a key role in regulating Rho GTPase expression in DCs and DC priming of T lymphocytes.

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Introduction

As professional antigen-presenting cells, dendritic cells (DCs) are essential initiators of adaptive immunity (Banchereau and Steinman, 1998; Shortman and Liu, 2002). DCs have several special features that contribute to efficient antigen presentation including high expression levels of MHC and costimulatory molecules as well as specialized morphology (Banchereau and Steinman, 1998; Shortman and Liu, 2002). DCs have membrane processes that are several times the length of their cell body. These processes, referred to as dendrites, interact with T lymphocytes to establish stable DC–T cell contact (Benvenuti *et al*, 2004). The small guanosine triphosphatases (GTPases) of the Rho family regulate multiple functions in DCs. Inhibition of Rac1 or Cdc42 activation by their dominant negative mutants results in

impaired antigen presentation and T-cell priming (Swetman *et al*, 2002; Kerksiek *et al*, 2005; Shurin *et al*, 2005). DCs lacking Rac1 and Rac2 expression fail to move toward T lymphocytes to establish a stable DC–T cell interaction (Benvenuti *et al*, 2004). These DCs are defective in priming CD4⁺ T cells *in vivo* and *in vitro*. In addition, several studies have shown that Rho GTPases are critically involved in DC endocytosis (Garrett *et al*, 2000; West *et al*, 2000; Kerksiek *et al*, 2005). However, it remains unclear how the expression of Rho GTPases is regulated in DCs.

Integrins are transmembrane receptors comprised of α and β heterodimers and recognize extracellular matrix (ECM) proteins including fibronectin, vitronectin, collagen and laminin as their ligands (Plow *et al*, 2000; Hynes, 2002). DCs express various types of integrins and some integrins have been used to define DC subsets (Kilshaw, 1993; Puig-Kroger *et al*, 2000; Pribila *et al*, 2004). Integrins such as α_4 , α_5 and β_1 are highly expressed by all mouse DCs (Pribila *et al*, 2004). Signaling through integrins activates Rho GTPases. For example, fibroblasts placed on fibronectin induced activation of Cdc42, Rac1 and Rho (Price *et al*, 1998; Ren *et al*, 1999). These studies suggest that an ECM protein/integrin/Rho GTPase signaling pathway may exist to regulate DC function. However, it is unknown which ECM protein–integrin interaction plays such a role in DCs.

Mindin (spondin 2) is a highly conserved ECM protein expressed abundantly in the spleen and lymph nodes (LNs) (He *et al*, 2004). It belongs to the mindin–F-spondin family that includes F-spondin and mindin (Klar *et al*, 1992; Higashijima *et al*, 1997; Umemiya *et al*, 1997; Feinstein *et al*, 1999). Mindin and F-spondin share three structural domains: an FS1 and FS2 (named for F-spondin) domain at the amino terminus as well as one or six thrombospondin repeats at the carboxyl terminus. Mindin matrix promotes neurite outgrowth from embryonic hippocampal neurons (Feinstein *et al*, 1999). Mindin also functions as a pattern recognition molecule for microbial pathogens (He *et al*, 2004). Mindin^{-/-} mice have an impaired capability to clear bacterial infection. Mindin^{-/-} macrophages exhibit defective responses to a broad spectrum of microbial stimuli. Our recent data also show that mindin interacts with integrins $\alpha_M\beta_2$ and $\alpha_4\beta_1$ on neutrophils and plays a critical role in inflammatory cell recruitment (Jia *et al*, 2005).

In this report, we show that mindin^{-/-} mice exhibited defective CD4⁺ T-cell priming and humoral response to T-dependent (TD) antigens. Mindin^{-/-} DCs failed to efficiently prime T lymphocytes *in vitro* owing to inefficient engagement with T cells and had reduced expression of Rac1, Rac2 and Cdc42. The defective priming capacity of mindin^{-/-} DCs was restored by ectopic expression of Rac1. Furthermore, we found that mindin interacts with $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins on DCs. DCs from β_1 integrin-deficient mice also exhibited reduced expression of Rac1 and Rac2 and defective priming of T lymphocytes. Collectively, our results

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have identified mindin as a key regulator of Rho GTPase expression in DCs and suggest that mindin-integrin interactions are essential for the normal expression of Rho GTPases in DCs.

Results

Humoral immune response in *mindin*^{-/-} mice

To examine the role of mindin in the adaptive immune response, we immunized *mindin*^{-/-} mice with sheep red blood cells (SRBCs), a TD antigen commonly used to study antibody responsiveness. As expected, SRBC immunization of wild-type mice elicited a strong specific IgG response (Figure 1A). In contrast, *mindin*^{-/-} mice exhibited an impaired anti-SRBC IgG production (Figure 1A). At the peak of the response 2–3 weeks after immunization, the anti-SRBC IgG amount in *mindin*^{-/-} mice was only ~5% of that of controls (Figure 1A). Furthermore, secondary immunization with SRBCs also displayed defective antibody production in *mindin*^{-/-} mice (Figure 1A).

We examined germinal center (GC) formation in the spleen of *mindin*^{-/-} mice immunized with SRBCs. As expected, GCs were readily detected in control mice 10 days after SRBC immunization (Figure 1B). In contrast, the size and number of GCs in the spleen of *mindin*^{-/-} mice were dramatically reduced as reflected by PNA and anti-FDC1 staining (Figure 1B). These results demonstrate that mindin plays a critical role in antibody production and GC formation after immunization with a TD antigen.

We also tested antibody production in *mindin*^{-/-} mice using another TD antigen, NP₂₄-KLH. As NP-KLH induces a strong IgG1 response (Croix *et al*, 1996), we tested the level of anti-NP IgG1 and the relative affinity for NP using different NP-BSA substrates. The total anti-NP₂₄ IgG1 level in both primary and secondary responses in *mindin*^{-/-} mice was reduced by 80% when compared to that of control mice (Figure 1C). Furthermore, although high-affinity anti-NP₆ IgG1 was detected in the mutant mice, the level was only one-third of that found in controls (Figure 1C). Given that defective antibody response in some mutant mice can be

Figure 1 Impaired humoral immune response to TD antigens in *mindin*^{-/-} mice. (A) Anti-SRBC-specific IgG in *mindin*^{-/-} (-/-) (circle, all throughout) and control (+/+) (square, all throughout) mice. Mice (*n* = 6) at 6–8 weeks of age were immunized intraperitoneally with 1×10^8 SRBC at day 0 and 28 and the sera were collected at the indicated time. Relative anti-SRBC IgG titers were determined by ELISA. (B) GC formation in SRBC-immunized *mindin*^{-/-} (-/-) and control (+/+) mice. Spleens from *mindin*^{-/-} and control mice 10 days after SRBC immunization were sectioned and stained as indicated. (C, D) Anti-NP-specific IgG1 in *mindin*^{-/-} and control mice. Mice (*n* = 5) at 6–8 weeks of age were immunized with NP₂₄-KLH (50 μg) alone (C) or NP₂₄-KLH (50 μg) plus alum (D) at days 0 and 28. Specific anti-NP IgG1 in the sera was determined by ELISA using NP₂₄-BSA or NP₆-BSA as substrates. (E, F). Antibody response to TI antigens. *Mindin*^{-/-} and control mice (*n* = 5) were immunized with TNP-LPS (50 μg) (E) or TNP-Ficoll (50 μg) (F) at day 0 and the sera were collected at a weekly interval. Serum anti-TNP antibody was determined by ELISA. Data are representative of 3–4 independent experiments.

restored by adjuvant (Wu *et al*, 2000), we tested antibody production in *mindin*^{-/-} mice immunized with NP₂₄-KLH precipitated with alum. Utilization of alum partially corrected the defective anti-NP antibody production in *mindin*^{-/-} mice (Figure 1D).

We next determined humoral immune response to T-independent (TI) antigens in *mindin*^{-/-} mice using the type I TI antigen TNP-LPS or the type 2 TI antigen TNP-Ficoll by i.p. injection. Antibody production was evaluated at different time points. *Mindin*^{-/-} mice produced similar amounts of IgM and IgG antibodies against both types of TI antigens to those in control mice (Figure 1E and F). Together, these results demonstrate that antibody responses to TD but not TI antigens were impaired and suggest that B lymphocyte response is normal in *mindin*^{-/-} mice.

Mindin expression in lymphocytes and DCs

The impaired TD antibody production in *mindin*^{-/-} mice suggests a possible defect in the interactions between T-B lymphocytes and/or T-DCs. Our previous data showed that *mindin* is abundantly expressed in macrophages and mast cells (He *et al*, 2004). To characterize the role of *mindin* in TD humoral immunity, we examined *mindin* expression in lymphocytes and DCs. CD4⁺ T cells, CD8⁺ T cells and B220⁺ B cells were purified from the spleen and lymph node (LN) of wild-type mice by FACS sorting and subjected to semiquantitative RT-PCR analysis. *Spondin2* (*mindin*) mRNA was readily detected in all subsets of lymphocytes (Figure 2A). In addition, *mindin* protein was readily detected in BM-derived DCs (BMDCs) from wild-type but not *mindin*^{-/-} mice (Figure 2B).

We further examined the expression pattern of *mindin* protein in secondary lymphoid organs by immunohistochemical staining. *Mindin* protein was detected in a constant and diffuse pattern in wild-type spleen with positive signals seen across the whole tissue section including both white and red pulps (Figure 2C). As expected, no signal was detected in *mindin*^{-/-} spleen after immunohistochemical staining (Figure 2C). These results demonstrate that *mindin* is expressed by mature lymphocytes and DCs in the secondary lymphoid organs.

In vivo CD4⁺ T-cell priming

The impaired humoral immune response to TD but not TI antigens in *mindin*^{-/-} mice may be owing to defective T-cell activation and/or priming. We first examined T- and B-cell proliferation by stimulating splenocytes with anti-CD3 or LPS *in vitro*. The proliferation of *mindin*^{-/-} T and B lymphocytes induced by anti-CD3 or LPS was essentially indistinguishable from that of control cells (Figure 2D), suggesting that *mindin* is not essential for T- or B-cell proliferation.

We then examined *in vivo* CD4⁺ T-cell priming in *mindin*^{-/-} mice. CD4⁺ T cells from the spleen and LNs of *mindin*^{-/-} and wild-type mice immunized with ovalbumin (Ova) plus LPS emulsified in incomplete Freund's adjuvant (Pasare and Medzhitov, 2004) were purified and restimulated *in vitro* with wild-type APCs. Proliferation of primed CD4⁺ T cells was measured by ³H-thymidine uptake. The proliferation of *in vivo* primed CD4⁺ T cells from *mindin*^{-/-} mice was 60–70% lower than that of control CD4⁺ T cells (Figure 2E). These results indicate that efficient priming of CD4⁺ T cells *in vivo* depends on *mindin*.

Figure 2 Impaired CD4⁺ T-cell priming in *mindin*^{-/-} mice. (A) *Spondin2* (*mindin*) mRNA expression in T and B lymphocytes. Total RNA from FACS sorted wild-type CD4⁺ and CD8⁺ T lymphocytes and B220⁺ B lymphocytes (>99%) was analyzed for *Spondin2* mRNA expression in semiquantitative RT-PCR. Samples were tested in 1:5 serial dilutions. *Hprt*, hypoxanthine phosphoribosyltransferase, serves as loading control. (B) *Mindin* protein expression in BMDCs. Total cell lysates (10 µg protein) from BMDCs of wild-type (+/+) and *mindin*^{-/-} (-/-) mice were blotted with a polyclonal anti-*mindin* antibody. (C) *Mindin* expression pattern in the spleen. Frozen spleen sections from wild-type (+/+) and *mindin*^{-/-} (-/-) mice were stained with polyclonal anti-*mindin* followed by HRP-labeled anti-Rabbit Ig and 3-aminoethylcarbrazole, and counterstained with methyl blue. (D) *In vitro* proliferation of T and B lymphocytes. Splenocytes from *mindin*^{-/-} (circle) and wild-type (square) mice were stimulated with anti-CD3 or LPS at the indicated concentrations for 48 h and pulsed with ³H-thymidine in the last 8 h of the culture. Mean of triplicate determination with s.d. <5% of the means are shown. (E) Proliferation of *in vivo* primed CD4⁺ T cells from *mindin*^{-/-} (-/-) and wild-type (+/+) mice. CD4⁺ T cells from mice immunized with Ova (50 µg) plus LPS (5 µg) in IFA for 10 days were stimulated with Ova (5 µg) presented by irradiated wild-type splenocytes for 3 days. Cells were pulsed with ³H-thymidine in the last 12 h of the culture. Mean + s.d. of triplicate determination are shown. Data are representative of three experiments.

Characterization of DCs in *mindin*^{-/-} mice

The normal TCR-mediated proliferation but defective *in vivo* priming of *mindin*^{-/-} T lymphocytes suggests that DCs may

have impairment in development and/or function in the absence of mindin. We first characterized DC development in mindin^{-/-} mice. The number of CD11c⁺ DCs in the spleen of mindin^{-/-} mice was comparable to that in age-matched control mice (Supplementary Figure 1A). Furthermore, the major DC subsets as defined by the expression of CD4 and CD8 on CD11c⁺ DCs (Kamath *et al*, 2000) were similarly detected in mindin^{-/-} and control mice (Supplementary Figure 1B). These results suggest that DC development is normal in mindin^{-/-} mice.

Mindin^{-/-} macrophages exhibit defective activation upon stimulation by microbial components (He *et al*, 2004). We examined whether mindin^{-/-} DCs have defects in the up-regulation of costimulatory molecules upon microbial component stimulation. Similar to control cells, both BM-derived and splenic DCs from mindin^{-/-} mice readily upregulated CD40, CD80 and CD86 as well as MHC class II upon LPS stimulation (Supplementary Figure 1C). Furthermore, BMDCs from mindin^{-/-} mice displayed similar survival kinetics with or without LPS stimulation (Supplementary Figure 1D). To determine whether mindin regulates DC endocytosis, we incubated immature BMDCs from mindin^{-/-} and control mice with FITC-Ova. Mindin^{-/-} DCs endocytosed FITC-Ova at a comparable level to that of control cells (Supplementary Figure 1E). Together, these results demonstrate that mindin is not involved in DC costimulatory molecule expression, survival or endocytosis. Given that mindin^{-/-} macrophages exhibited defective phagocytosis of bacteria, we further examined the phagocytosis capacity of mindin^{-/-} DCs. Consistent with previous results (He *et al*, 2004), mindin^{-/-} DCs exhibited defective phagocytosis of *Streptococcus pneumoniae* and *Salmonella typhimurium*, but normal FcR-mediated phagocytosis of SRBCs (Supplementary Figure 2).

Impaired priming capacity by mindin^{-/-} DCs

To directly test the priming capacity of mindin^{-/-} DCs, we set up *in vitro* DC-T cell priming cultures by mixing splenic DCs with naïve CD4⁺ T cells in the presence of Ova. Wild-type DCs induced similarly high levels of proliferation in naïve CD4⁺ T cells from wild-type and mindin^{-/-} mice (Figure 3A),

suggesting that mindin^{-/-} T cells have the ability to respond to DC priming. In contrast, mindin^{-/-} DCs induced only low levels of proliferation in mindin^{-/-} CD4⁺ T cells (Figure 3A). Interestingly, mindin^{-/-} DCs induced an intermediate level of proliferation in wild-type T cells (Figure 3A), suggesting that mindin produced by wild-type T cells may partially restore the priming function of mindin^{-/-} DCs.

We then used OTII CD4⁺ T cells expressing a transgenic TCR recognizing an Ova peptide (Barnden *et al*, 1998) in the DC-T cell priming culture. Mindin^{-/-} DCs induced an intermediate level of proliferation in OTII CD4⁺ T cells when compared to control DCs, consistent with the above results using wild-type T cells (Figure 3B). Furthermore, the defective priming capacity was also seen in BMDCs from mindin^{-/-} mice (Figure 3C) and could be completely restored by exogenous mindin at 10 µg/ml (Figure 3D). These results demonstrate that mindin^{-/-} DCs are defective in priming T lymphocytes *in vitro*.

We determined whether preincubating mindin with either DCs or T cells can restore the DC priming defect. Interestingly, preincubation of recombinant mindin with T cells or mindin coated on plates during DC-T cell culture restored the defective priming of mindin^{-/-} DCs, whereas preincubation of mindin with DCs failed to do so (Figure 3E). These results suggest that mindin binds to T cells. The failure to restore DC priming after mindin preincubation with DCs may reflect that the bound mindin may be endocytosed by DCs.

Mindin^{-/-} macrophages produce lower levels of IL-6 than wild-type cells when stimulated by microbial components (He *et al*, 2004). Recent studies suggest that IL-6 plays a critical role in T-cell priming by blocking the suppressive effect of Treg cells (Pasare and Medzhitov, 2003, 2004). *In vivo* depletion of Treg cells restored defective T-cell priming in MyD88^{-/-} mice (Pasare and Medzhitov, 2004). We found that LPS-stimulated mindin^{-/-} DCs produced only ~40% of IL-6 and TNF-α when compared to control DCs (data not shown). To test whether a reduced production of IL-6 may account for the impaired priming capacity of mindin^{-/-} DCs, we added exogenous IL-6 to *in vitro* DC-T cell culture. Excess amount of IL-6 in the DC-T cell priming culture failed to restore the priming capacity of mindin^{-/-} DCs (Figure 4A).

Figure 3 Impaired priming capacity of mindin^{-/-} DCs. Purified naïve CD4⁺ T cells and mature DCs from either mindin^{-/-} (-/-) or wild-type (+/+) mice were cocultured as indicated in the presence of Ova for 3–5 days. CD4⁺ T-cell proliferation was measured by ³H-thymidine uptake. The following cells were used: (A, B) splenic DCs from mindin^{-/-} and wild-type mice; (C–E) BMDCs from mindin^{-/-} and wild-type mice; (A, C–E) CD4⁺ T cells from mindin^{-/-} and wild-type mice; (B) CD4⁺ T cells from OTII transgenic mice. (D) Restoration of priming capacity of mindin^{-/-} DCs by exogenous mindin. DC-T cell coculture was set up as in (A). Exogenous mindin was added at 10 µg/ml. (E) Restoration of priming capacity of mindin^{-/-} DCs by different ways of treatment with mindin. Mindin (10 µg/ml) was either preincubated with DCs or T cells at 37°C for 1 h and washed three times, precoated on the plates, or added to the culture medium. Mean of triplicate determinations with s.d. <10% of the means are shown and are representative of 3–5 experiments.

Figure 4 Mechanistic examination of the impaired priming capacity of mindin $^{-/-}$ DCs. (A) Effect of IL-6 on *in vitro* T-cell priming by mindin $^{-/-}$ DCs. CD4 $^+$ OTII T cells were *in vitro* primed by BMDCs from mindin $^{-/-}$ ($-/-$) or wild-type ($+/+$) mice with or without IL-6 (10 U/ml). (B) Effect of *in vivo* depletion of Treg cells on T-cell priming in mindin $^{-/-}$ mice. Mindin $^{-/-}$ mice treated with anti-CD25 mAb or control rat Ig at day -3 were immunized with Ova (50 μg) plus LPS (5 μg) in IFA at day 0. CD4 $^+$ T cells were isolated at day 10 and restimulated with Ova at the indicated concentration presented by irradiated wild-type splenocytes. Wild-type mice treated with rat Ig and similarly immunized serve as positive control for *in vitro* priming efficiency. (C) Priming capacity of mindin $^{-/-}$ DCs matured by TNF- α stimulation. BMDCs from mindin $^{-/-}$ ($-/-$) and control ($+/+$) mice were stimulated with TNF- α (50 ng/ml) for 16 h before used in *in vitro* priming. (D) Defective priming of T cells by mindin $^{-/-}$ DCs in the presence of Ova peptide. BMDCs from mindin $^{-/-}$ ($-/-$) and control ($+/+$) mice were cocultured with OTII CD4 $^+$ T cells in the presence of Ova₃₂₃₋₃₃₉ peptides as indicated. (E) Mixed lymphocyte reaction induced by mindin $^{-/-}$ DCs. Irradiated BMDCs from mindin $^{-/-}$ ($-/-$) and control ($+/+$) mice (C57BL/6 background) ($1 \times 10^6/\text{ml}$) were cultured with naïve CD4 $^+$ T cells from Balb/c mice at the indicated concentrations. The proliferation of CD4 $^+$ T cells were measured by ^3H -thymidine uptake. Mean of triplicate determinations with s.d. < 10% of the means are shown and are representative of 3–4 experiments.

Furthermore, *in vivo* depletion of Treg cells in mindin $^{-/-}$ mice did not restore T-cell priming (Figure 4B). These results suggest that the defective antigen presentation of mindin $^{-/-}$ DCs is not solely due to reduced IL-6 production or an inability to overcome suppression by Treg cells *in vivo*.

The defective priming capacity of mindin $^{-/-}$ DCs may be solely due to their impaired maturation induced by LPS. To address this, mindin $^{-/-}$ DCs matured by TNF- α stimulation were tested for their priming capacity. A similar priming defect of mindin $^{-/-}$ DCs matured by TNF- α to that of DCs matured by LPS was observed (Figure 4C). Furthermore, mindin $^{-/-}$ DCs failed to prime T cells efficiently even when Ova peptides were used as the antigen (Figure 4D), arguing against that the priming defect is owing to impaired antigen uptake, processing and MHC peptide loading. Interestingly, mindin $^{-/-}$ DCs stimulated allogeneic CD4 $^+$ T cells as efficiently as control DCs in a mixed lymphocyte reaction (Figure 4E), indicating that allostimulation by DCs does not depend on mindin.

Rac1/2 expression in mindin $^{-/-}$ DCs

Given the role of Rac1 and Rac2 in regulating DC priming capability (Benvenuti *et al*, 2004) and the fact that mindin serves as a ligand for integrins (Jia *et al*, 2005), we examined whether mindin regulates the expression of these small GTPases in DCs. Total cell lysates of purified splenic DCs from mindin $^{-/-}$ and wild-type mice were blotted for total Rac1, Rac2 and Cdc42 expression. Unstimulated splenic DCs from wild-type mice expressed abundant levels of these GTPases (Figure 5A). In contrast, the expression of Rac1, Rac2 and Cdc42 was much lower in mindin $^{-/-}$ splenic DCs (Figure 5A). Furthermore, whereas microbial component stimulation readily increased the expression levels of these Rho GTPases in wild-type DCs, less upregulation was observed in mindin $^{-/-}$ DCs (Figure 5A). Consistent with their defective priming capacity, the expression and upregulation of Rac1, Rac2 and Cdc42 were also impaired in mindin $^{-/-}$ BMDCs (Figure 5B).

We next determined whether the reduced Rac1/2 expression in mindin $^{-/-}$ DCs was owing to a lowered mRNA expression using quantitative RT-PCR. Expression of Rac1 and Rac2 mRNA in unstimulated splenic DCs from mindin $^{-/-}$ mice was only ~40% of that found in wild-type DCs (Figure 5C). Furthermore, the lower levels of Rac1 and Rac2 mRNA expression in mindin $^{-/-}$ DCs was not restored by LPS or PGN (peptidoglycan) stimulation (Figure 5C), suggesting that mindin is required for normal expression of Rac1 and Rac2 mRNA in DCs.

It has been reported that DCs lacking Rac1/2 expression fail to prime T cells owing to inefficient DC-T cell contact and the defect can be restored by coculturing DCs and T cells in round-bottom plates (Benvenuti *et al*, 2004). If the reduced Rac1/2 expression in mindin $^{-/-}$ DCs is the primary defect, DC priming in round-bottom plates may restore T-cell proliferation. Indeed, T-cell priming by mindin $^{-/-}$ DCs was completely restored when round-bottom plates were used (Figure 5D). To further determine which Rho GTPase, Rac or Cdc42, accounts for the defective priming capacity of mindin $^{-/-}$ DCs, we ectopically expressed Rac1 or Cdc42 in BMDCs of mindin $^{-/-}$ and wild-type mice using the pMI retrovirus vector expressing human CD2 as a marker (He *et al*, 1998). Human CD2 $^+$ BMDCs were purified by FACS sorting and incubated with OTII CD4 $^+$ T cells in the presence of Ova. Expression of Rac1 (and human CD2) in wild-type DCs did not obviously affect their antigen presentation to OTII CD4 $^+$ T cells (Figure 5E). Expression of Rac1 in mindin $^{-/-}$ DCs completely restored T-cell priming (Figure 5E). In contrast, expression of Cdc42 in mindin $^{-/-}$ DCs failed to restore their priming capacity (Figure 5E). In addition, Cdc42 expression in wild-type DCs appeared to inhibit their antigen presentation to OTII CD4 $^+$ T cells (Figure 5E). Consistent with the restoration of mindin $^{-/-}$ DC priming by recombinant mindin, the expression of Rac1 and Cdc42 in mindin $^{-/-}$ DCs was also restored by exogenous mindin (Figure 5F and data not shown). Collectively, these results demonstrate that mindin plays a critical role in the normal expression and

Figure 5 Reduced Rac1/2 expression in mindin^{-/-} DCs. (A, B) Expression of Rac1, Rac2 and Cdc42 in splenic DCs (A) and BMDCs (B) from mindin^{-/-} (-/-) and wild-type (+/+) mice. Total cell lysates from untreated DCs (None) or DCs stimulated with LPS (60 ng/ml), zymosan, LTA and PGN (all 10 μg/ml) for 12 h were blotted with the indicated antibodies. ERK protein expression serves as a loading control. (C) Rac1 and Rac2 mRNA expression in mindin^{-/-} DCs. Total RNA from splenic DCs of mindin^{-/-} (-/-) and wild-type (+/+) mice with or without stimulation were analyzed for Rac1/2 mRNA expression by real-time PCR. The relative mRNA levels of Rac1 and Rac2 were normalized for *Gapdh* mRNA expression and expressed as 100% for wild-type DCs. Data are mean ± s.d. of triplicate determinations. (D) Restoration of priming capacity of mindin^{-/-} DCs with round-bottom culture plates. DC-T cell priming was carried out as in Figure 4C except that round-bottom plates were used. (E) Restoration of priming capacity of mindin^{-/-} DCs by retrovirally delivered Rac1. BMDCs expressing Rac1 or Cdc42 from mindin^{-/-} (-/-) and wild-type (+/+) mice were sorted by FACS and used as APCs in DC-OT II CD4⁺ T-cell priming culture in the presence of Ova (5 μg/ml). The proliferation of OT II T cells was measured by ³H-thymidine uptake in the last 12 h of the culture. Mean ± s.d. of triplicate determinations are shown. Data are representative of three experiments. (F) Restoration of Rac1 expression in mindin^{-/-} DCs by recombinant mindin. BMDCs from mindin^{-/-} (-/-) and control (+/+) mice were incubated with mindin (10 μg/ml) for 16 h and analyzed for Rac 1 expression by Western blot. Loading control is a nonspecific band on the same membrane.

upregulation of Rho GTPases in DCs and that the reduced Rac expression accounts for the defective priming capacity in mindin^{-/-} DCs.

Defective interaction between Mindin^{-/-} DCs and T lymphocytes

To directly visualize the interaction between mindin^{-/-} DCs and T lymphocytes *in vitro*, we performed real-time imaging

of DC:T interaction using *in vitro* cell coculture. Wild-type DCs formed stable clusters with OTII CD4⁺ T cells as early as 2 h (Supplementary movie). Multiple T cells attached readily to wild-type DCs, and this attachment persisted for the observed period (Supplementary movie). In contrast, mindin^{-/-} DCs only transiently interacted with OTII T cells and had markedly less OTII cells attached despite that these cells had normal appearance of dendrites (Supplementary movie). Furthermore, Mindin^{-/-} DCs displayed faster movement (Supplementary movie). We then tracked the movement of DCs in the coculture. As shown in Figure 6A, wild-type DCs rapidly engaged with OTII T cells and essentially remained at the same spot for 6 h, whereas mindin^{-/-} DCs failed to engage T cells and moved out of the imaging field within 90 min. The cumulative distance of mindin^{-/-} DC movement was increased dramatically compared to that of control DCs (Figure 6B), indicating that mindin^{-/-} DCs moved continuously whereas wild-type DCs stopped moving after engaging OTII T cells. Furthermore, the velocity of mindin^{-/-} DCs was 5–6-fold higher than that of control DCs (Figure 6C). Taken together, these results demonstrate that mindin^{-/-} DCs cannot engage T lymphocyte efficiently. The reduced movement and velocity of wild-type DCs are consistent with the observations that DCs form stable clusters with T cells during antigen presentation *in vivo* (Stoll *et al*, 2002; Bousso and Robey, 2003; Miller *et al*, 2004).

Mindin as a ligand for integrin on DCs

Our recent data show that mindin serves as a ligand for integrin $\alpha_M\beta_2$ and $\alpha_4\beta_1$ on neutrophils (Jia *et al*, 2005). Various integrins are expressed on mouse DCs and can be used to define subsets of DCs (Kilshaw, 1993; Puig-Kroger *et al*, 2000; Pribila *et al*, 2004). Given the uniformly high expression of $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins on all mouse DC subsets, we examined whether these integrins on DCs interact with mindin matrix. Immature and mature DCs readily adhered to mindin and fibronectin but not laminin matrix (Supplementary Figure 3A). Addition of anti- β_1 integrin mAb reduced DC adhesion to mindin matrix by 50–70% (Supplementary Figure 3A). Addition of anti- α_4 or anti- α_5 integrin mAbs also reduced DC adhesion to mindin matrix, though combination of these antibodies did not further reduce mindin-mediated DC binding (Supplementary Figure 3A). As a negative control, anti-FcR2/III mAb did not affect DC adhesion to mindin matrix (Supplementary Figure 3A). These results suggest that DCs utilize integrins $\alpha_4\beta_1$ and $\alpha_5\beta_1$ to interact with mindin matrix.

We wondered whether mindin regulates DC function through modulating integrin expression levels. We determined the expression of integrin β_1 , α_4 and α_5 on mature DC by FACS analysis and found that the expression of these integrins on mature DCs from mindin^{-/-} and control mice was almost identical (Supplementary Figure 3B), ruling out a role of mindin in regulating integrin expression on DCs.

Role of integrin β_1 in DC priming

The above results suggest that mindin may regulate Rac1/2 expression in DCs by signaling through $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins. To test this, we examined Rac1/2 expression in DCs lacking β_1 integrin expression. To generate DCs lacking β_1 integrin expression, we crossed mice with β_1 integrin alleles flanked with two loxP sites (β_1 f/f) (Raghavan *et al*, 2000) to Mx-cre

Figure 6 Real-time observation of the interaction between mindin^{-/-} DC and T lymphocytes. **(A)** Representative cell centroid tracks of wild-type (+/+) (red) (a-f) and mindin^{-/-} (green) (g-l) BMDCs. Strip chart time lapse movies to follow the centroid tracks of one DC each from wild-type and mindin^{-/-} mice are shown. Time scale a-f is 354 min, g-l is 90 min, with each of the two strip charts divided into time points spaced at exact sixth of the total time in each experiment. Each individual panel is 86 μm wide. **(B)** Cumulative distance of DC movement in DC-T cell coculture. DCs from each group were randomly picked and tracked for their movement until the cells moved out of the imaging field. Wild-type DCs rarely moved out whereas mindin^{-/-} DCs all moved out of the tracking field after 90 min. The mean values of 15 randomly tracked DCs from each group are shown. **(C)** Velocity of splenic and BM-derived DCs from wild-type and mindin^{-/-} mice in DC-T cell coculture. Data are mean $\pm$ s.d. calculated from the same 15 cells in (B) and representative of three independent experiments. *P*-values between +/+ and -/- groups are <0.001 .

transgenic mice (Kuhn *et al*, 1995) and induced β_1 integrin deletion by repetitively injecting polyI:C. Mx-cre induced an efficient deletion of β_1 integrin on ~ 40 – 50% splenic DCs as assessed by FACS (Figure 7A). We purified $\beta_1^{\text{low/neg}}$ splenic DCs from β_1/f Mx-cre mice by FACS sorting. Sorted splenic DCs from β_1/f mice similarly treated with polyI:C were used as a control (Figure 7A). We first tested their binding to mindin matrix. The binding of β_1 integrin-deficient DCs to mindin was reduced by $\sim 40\%$ when compared to that of control DCs (Figure 7B), suggesting that β_1 integrin mediates mindin-DC interaction. We then examined Rac1 and Rac2

expression in these DCs. The expression levels of total Rac1 and Rac2 proteins in β_1 -deficient splenic DCs were reduced by ~ 70 and $\sim 40\%$, respectively, when compared to control DCs (Figure 7C). BMDCs from β_1/f Mx-cre mice, which had efficient β_1 integrin deletion on $>90\%$ of the cells, contained a dramatically reduced levels of Rac1 and Rac2 (Figure 7C). Furthermore, upregulation of Rac1 and Rac2 expression upon microbial component stimulation in β_1 -deficient splenic DCs and BMDCs was also impaired (Figure 7C). Correlated with the lower Rac1 and Rac2 protein expression in β_1 integrin-deficient DCs, Rac1 and Rac2 mRNA expression levels were

Figure 7 Impaired antigen presentation of β_1 (CD29)-deficient DCs. **(A)** FACS profiles of sorted β_1 -deficient splenic DCs. Splenic DC preparations from β_1/fMx -cre ($-/-$) and $\beta_1/f/f$ (f/f) mice treated with polyI:C were stained with anti-CD11c and anti-CD29 and subjected to FACS sorting. These sorted β_1 -deficient and β_1^+ splenic DCs were used in the experiments shown below. **(B)** Binding of β_1 -deficient splenic DCs to mindin matrix. LPS matured β_1 -deficient ($-/-$) and control ($+/+$) splenic DCs as shown in (A) were incubated with precoated mindin (10 $\mu\text{g/ml}$) at room temperature for 1 h, washed and counted. Data are mean $\pm$ s.d. from at least four different fields. **(C)** Expression of Rac1/2 in β_1 -deficient DCs. Total cell lysates from splenic or BM-derived DCs of β_1/fMx -cre ($-/-$) and $\beta_1/f/f$ (f/f) mice without treatment (None) or stimulated with LPS (60 ng/ml), LTA, PGN, zymosan, mannan (all 10 $\mu\text{g/ml}$) for 12 h were blotted with anti-Rac1 or anti-Rac2. Loading control is a nonspecific band from the same membrane. **(D)** Rac1 and Rac2 mRNA expression in β_1 integrin-deficient DCs. Total RNA from sorted splenic DCs of β_1/fMx -cre ($-/-$) and wild-type ($+/+$) mice treated with polyI:C were analyzed for Rac1/2 mRNA expression by real-time PCR. The relative mRNA levels of Rac1 and Rac2 were normalized for *Gapdh* mRNA expression and expressed as 100% for wild-type DCs. Data are mean $\pm$ s.d. of triplicate determinations. **(E, F)** Impaired antigen presentation by β_1 integrin-deficient DCs. Integrin β_1^- or β_1^+ splenic (E) or BM-derived DCs (F) from β_1/fMx -cre ($-/-$), $\beta_1/f/f$ (f/f) or $\beta_1^{+/+}$ ($+/+$) mice treated with polyI:C were incubated with OTII CD4⁺ T cells in the presence of ovalbumin for 3 days. Mean of triplicate determinations with s.d. $<10\%$ of the mean are shown. **(G)** Effect of ectopic Rac1 expression on antigen presentation of β_1 integrin-deficient DCs. BMDCs from β_1/fMx -cre, $\beta_1/f/f$ or $\beta_1^{+/+}$ wild-type mice infected with pMIRac1 (Rac1) or pMI (Vector) retroviruses were sorted by FACS and incubated with OTII CD4⁺ T cells in the presence of ovalbumin (5 $\mu\text{g/ml}$). Mean $\pm$ s.d. of triplicate determinations are shown. Data are representative of 2–4 experiments.

also reduced in these cells when compared to wild-type DCs (Figure 7D). These results indicate that β_1 integrin-mediated signaling plays a critical role in Rac expression in DCs.

We then examined the priming capacity of these DCs in DC–T cell coculture. Similar to mindin $^{-/-}$ DCs, β_1 integrin-deficient splenic DCs and BMDCs displayed defective priming of OTII CD4⁺ T cells *in vitro* (Figure 7E and F). The reduced expression of Rac proteins and impaired priming capability in β_1 integrin-deficient DCs was not owing to a nonspecific effect caused by polyI:C treatment as wild-type and $\beta_1/f/f$ mice were also treated with polyI:C. Given the reduced expression of Rac1/2 in β_1 -deficient DCs, we tested whether ectopically expressed Rac1 could restore the defective antigen presentation in these cells. Interestingly, Rac1 expression did not restore the defective antigen presentation of β_1 -deficient DCs (Figure 7G). Collectively, these results demonstrate that

integrin β_1 plays a critical role in the expression of Rac1/2 and antigen presentation capability of DCs.

Discussion

In this report, we show that mindin $^{-/-}$ mice have defective humoral immune responses to TD but not TI antigens. Mindin was first characterized as a pattern recognition molecule for microbial pathogens through carbohydrate recognition and is essential for the initiation of innate immune response to microbial pathogens (He *et al*, 2004). We have used nonpathogenic antigens to examine adaptive immune response in mindin $^{-/-}$ mice. Given that mindin is required for IL-6 and TNF- α production and inflammatory cell recruitment (He *et al*, 2004; Jia *et al*, 2005), it is likely that *in vivo* defects in migration of DCs or lymphocytes and/or lack of

inflammatory cytokine production also contribute to the defective humoral immune response in *mindin*^{-/-} mice. Nevertheless, our *in vitro* data supports a role for *mindin* in DC-T cell priming by maintaining Rho GTPase expression in DCs.

As professional APCs, DCs make close interaction with T lymphocytes to establish a stable contact (Benvenuti *et al* (2004) and Supplementary movie). Without this stable contact, DC priming of T lymphocytes is impaired. Recent reports have unequivocally demonstrated that Rho GTPases control DC priming capability (Benvenuti *et al*, 2004; Kerkisiek *et al*, 2005). Several lines of evidence suggest that the impaired priming capacity of *mindin*^{-/-} DCs is owing to reduced expression of Rac1 and Rac2. First, Rac 1 and Rac 2 expression levels were reduced in immature splenic, mature splenic and BM-derived DCs in *mindin*^{-/-} mice. Ectopic expression of Rac1, but not Cdc42, restored the priming capability of *mindin*^{-/-} DCs *in vitro*. Second, similar to DCs lacking both Rac1/2 expression (Benvenuti *et al*, 2004), coculture of *mindin*^{-/-} DCs with T lymphocytes in round-bottom plates completely restored the impaired priming capacity of *mindin*^{-/-} DCs. These results suggest that *mindin*^{-/-} DCs cannot interact with T lymphocytes closely. Furthermore, real-time imaging studies demonstrate that *mindin*^{-/-} DCs cannot engage T lymphocytes efficiently. Third, *mindin*^{-/-} DCs did not display any obvious defect in the upregulation of costimulatory molecules, endocytosis or survival. The fact that *mindin*^{-/-} DCs failed to efficiently prime T lymphocytes when Ova peptide was used as the antigen strongly argues against that the priming defect is caused by antigen uptake, processing and MHC peptide loading. Finally, *mindin*^{-/-} DCs matured by TNF- α stimulation still exhibited defective priming capacity, suggesting that the defect is not solely owing to a requirement on LPS by *mindin*^{-/-} DCs for their maturation.

There are notable differences in DC development and function between *mindin*^{-/-} and Rac1/2-deficient mice. CD8 α ⁺ DC development was impaired and dendrite formation was abnormal in Rac1/2-deficient mice (Benvenuti *et al*, 2004). In contrast, CD8 α ⁺ DC development was normal in *mindin*^{-/-} mice. Furthermore, we did not observe obvious defects in dendrite formation in *mindin*^{-/-} DCs (Supplementary Movie). The normal dendrite formation in *mindin*^{-/-} DCs may be owing to compensatory function from other ECM proteins such as fibronectin and collagens. The differences in CD8 α ⁺ DC development and dendrite formation in these two types of mice likely reflect the different expression levels of Rac1 and Rac2 in these mice. Our results suggest that the amounts of Rac1 and Rac2 in *mindin*^{-/-} DCs are sufficient for CD8 α ⁺ DC development and dendrite formation, but insufficient for efficient priming.

Our data suggest that *mindin* regulates Rho GTPase expression in DCs by interacting with $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins. We have recently shown that *mindin* serves as a ligand for $\alpha_M\beta_2$ and $\alpha_4\beta_1$ integrins on neutrophils (Jia *et al*, 2005). Given the high level expression of $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins on all DCs (Pribila *et al*, 2004) (Figure 7A), we focused our attention on these integrins. Our antibody blocking results indicate that DC adhesion to *mindin* matrix is mediated through $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins. This is further supported by the observation that β_1 integrin-deficient DCs exhibited reduced binding to *mindin* matrix. We also demonstrate that DCs lacking β_1 integrin expression exhibited reduced expression of Rac1/2

and impaired antigen presentation to T lymphocytes. The similarity between *mindin*^{-/-} and β_1 integrin-deficient DCs suggests that *mindin*- β_1 integrin interaction on DCs *in vivo* plays a key role in the expression of Rac1/2.

Our results indicate that DCs require constant interaction with environmental signals such as *mindin* to maintain normal function. At steady state, DCs are enmeshed in an extensive network, actively probing adjacent T lymphocytes with their dendrites in secondary lymphoid organs (Lindquist *et al*, 2004). Given the extensive expression of *mindin* in the extracellular matrix of secondary lymphoid organs, DCs must be in constant contact with *mindin*. This interaction is required for the normal expression of Rac proteins in DCs. This finding is surprising given that other $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrin ligands, such as fibronectin, are also expressed in these lymphoid organs (Liakka and Autio-Harminen, 1992; Lo *et al*, 2003). The fact that the interaction between fibronectin and these integrins is not sufficient to compensate for *mindin*-mediated integrin signaling suggests that *mindin* as a ligand provides a unique signal. It has been shown that $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins recognize RDG or other peptide motifs on fibronectin. It will be important to determine which motifs on *mindin* are recognized by these integrins.

We also show that a critical function of β_1 integrin in DCs is to directly regulate Rac1 and Rac2 mRNA expression through interaction with *mindin*. Upon interaction with ECM ligands, integrins regulate Rho GTPases function in several ways: integrin signaling readily activates Rac and Cdc42 from GDP-bound inactive form to GTP-bound active form (Price *et al*, 1998; Ren *et al*, 1999). Integrin signaling also promotes Rho GTPases to interact with effector proteins and their membrane localization (del Pozo *et al*, 2000, 2002). Our results demonstrate that β_1 integrin provides a critical signal to maintain normal expression levels of Rac1 and Rac2. This regulation occurs at transcription level as mRNA expression of Rac1 and Rac2 in both *mindin*^{-/-} and β_1 integrin-deficient DCs were lower than those found in wild-type DCs. However, it is possible that *mindin*-integrin interaction also regulates Rac expression at the protein level. Given the extensive studies on integrin-mediated signaling pathways (Hynes, 2002; Miranti and Brugge, 2002), it will be important to determine which signaling process mediated through β_1 integrin regulates Rac mRNA expression in DCs. In summary, our results suggest that *mindin*/integrin β_1 /Rac signaling process plays a critical role in DC priming of T lymphocytes.

Materials and methods

Mice

All homozygous *mindin*^{-/-} and wild-type mice were derived from breeding of heterozygous *mindin*^{+/-} mice (He *et al*, 2004) after back-crossed to C57BL/6 for seven generations. The following mice were obtained from the Jackson Laboratory: CD29 (β_1 integrin) floxed (CD29f/f) mice (Raghavan *et al*, 2000) and OTII TCR transgenic mice (Barnden *et al*, 1998). To generate β_1 integrin-deficient DC, CD29f/f mice were crossed with Mx-cre transgenic mice (Kuhn *et al*, 1995). The CD29f/fMx-cre and CD29f/f control mice at 5 weeks of age were injected with 300 μ g of polyI:C (Sigma) every other day for three times. Mice were then injected with the same amount of polyI:C 2 more times at 1-week intervals (five injections in total). All mice were housed in a specific pathogen-free facility at the Duke University Vivarium and used at 6–12 weeks of age. Mice in different groups were sex and age-matched. Animal usage was carried out according to protocols approved by the Duke University Institutional Animal Care and Use Committee.

DC preparation and stimulation

To isolate splenic DCs, spleens were perfused with serum-free RPMI containing Liberase CI (400 µg/ml) (Rache Diagnostics) at 37°C for 30 min. The digested spleens were lysed of erythrocytes and resuspended (1 ml/spleen) in a 30% BSA solution (Sigma). Cold RPMI (4 ml) was placed over the BSA/splenocyte mixture to form an interface. After centrifugation at 7000 r.p.m. for 15 min, the interface was harvested for enriched splenic DCs. To further purify CD11c⁺ DCs, enriched splenic DCs were stained with anti-CD11c mAb and sorted by FACS to >98% pure. BMDCs were generated by culturing BM precursor cells in the presence of GM-CSF (200 U/ml) and IL-4 (5 U/ml) (PeproTech) for 6 days as described (Inaba *et al*, 1992; Zhang *et al*, 2004). At the end of the culture, CD11c⁺ DCs accounted for 85–90% of the cells. All splenic DCs and BMDCs were pulsed with antigen, induced to undergo maturation by LPS stimulation (60 ng/ml) for 16 h and irradiated (3000 rads) before used for *in vitro* priming of T lymphocytes.

To express Rac1 or Cdc42 in DCs, the pMI retrovirus vector expressing human CD2 and mouse wild-type Rac1 and Cdc42 cDNA bicistronically were used to generate retroviruses as described (He *et al*, 1998). BMDCs were infected with retroviruses at day 2 and day 4 of the culture. Infected human CD2⁺ and CD11c⁺ BMDCs

were sorted by FACS, pulsed with antigen and induced to mature with LPS before being used in T-cell priming. Analysis of DC phagocytic capability using FITC-Ova was performed as described (Zhang *et al*, 2004).

DCs (1–2 × 10⁶ cells/ml) were stimulated with the following microbial component, LPS from *Salmonella typhosa* or *Escherichia coli* (Sigma), LTA from *S. aureus* (Sigma), zymosan and mannan from *Saccharomyces cerevisiae* (Sigma), and PGN from *S. aureus* (Fluka Chemical) at 37°C for 12 h. Total cell lysates were prepared for protein blot.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

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References

- Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. *Nature* **392**: 245–252
- Barnden MJ, Allison J, Heath WR, Carbone FR (1998) Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements. *Immunol Cell Biol* **76**: 34–40
- Benvenuti F, Hugues S, Walmsley M, Ruf S, Fetler L, Popoff M, Tybulewicz VL, Amigorena S (2004) Requirement of Rac1 and Rac2 expression by mature dendritic cells for T cell priming. *Science* **305**: 1150–1153
- Bouso P, Robey E (2003) Dynamics of CD8⁺ T cell priming by dendritic cells in intact lymph nodes. *Nat Immunol* **4**: 579–585
- Croix DA, Ahearn JM, Rosengard AM, Han S, Kelsoe G, Ma M, Carroll MC (1996) Antibody response to a T-dependent antigen requires B cell expression of complement receptors. *J Exp Med* **183**: 1857–1864
- Del Pozo MA, Kiosses WB, Alderson NB, Meller N, Hahn KM, Schwartz MA (2002) Integrins regulate GTP-Rac localized effector interactions through dissociation of Rho-GDI. *Nat Cell Biol* **4**: 232–239
- del Pozo MA, Price LS, Alderson NB, Ren XD, Schwartz MA (2000) Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK. *EMBO J* **19**: 2008–2014
- Feinstein Y, Borrell V, Garcia C, Burstyn-Cohen T, Tzarfaty V, Frumkin A, Nose A, Okamoto H, Higashijima S, Soriano E, Klar A (1999) F-spondin and mindin: two structurally and functionally related genes expressed in the hippocampus that promote outgrowth of embryonic hippocampal neurons. *Development* **126**: 3637–3648
- Garrett WS, Chen LM, Kroschewski R, Ebersold M, Turley S, Trombetta S, Galan JE, Mellman I (2000) Developmental control of endocytosis in dendritic cells by Cdc42. *Cell* **102**: 325–334
- He YW, Deftos ML, Ojala EW, Bevan MJ (1998) RORgamma t, a novel isoform of an orphan receptor, negatively regulates Fas ligand expression and IL-2 production in T cells. *Immunity* **9**: 797–806
- He YW, Li H, Zhang J, Hsu CL, Lin E, Zhang N, Guo J, Forbush KA, Bevan MJ (2004) The extracellular matrix protein mindin is a pattern-recognition molecule for microbial pathogens. *Nat Immunol* **5**: 88–97
- Higashijima S, Nose A, Eguchi G, Hotta Y, Okamoto H (1997) Mindin/F-spondin family: novel ECM proteins expressed in the zebrafish embryonic axis. *Dev Biol* **192**: 211–227
- Hynes RO (2002) Integrins: bidirectional, allosteric signaling machines. *Cell* **110**: 673–687
- Inaba K, Inaba M, Romani N, Aya H, Deguchi M, Ikehara S, Muramatsu S, Steinman RM (1992) Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* **176**: 1693–1702
- Jia W, Li H, He YW (2005) The extracellular matrix protein mindin serves as an integrin ligand and is critical for inflammatory cell recruitment. *Blood* **106**: 3854–3859
- Kamath AT, Pooley J, O'Keefe MA, Vremec D, Zhan Y, Lew AM, D'Amico A, Wu L, Tough DF, Shortman K (2000) The development, maturation, and turnover rate of mouse spleen dendritic cell populations. *J Immunol* **165**: 6762–6770
- Kerksiek KM, Niedergang F, Chavrier P, Busch DH, Brocker T (2005) Selective Rac1 inhibition in dendritic cells diminishes apoptotic cell uptake and cross-presentation *in vivo*. *Blood* **105**: 742–749
- Kilshaw PJ (1993) Expression of the mucosal T cell integrin alpha M290 beta 7 by a major subpopulation of dendritic cells in mice. *Eur J Immunol* **23**: 3365–3368
- Klar A, Baldassare M, Jessell TM (1992) F-spondin: a gene expressed at high levels in the floor plate encodes a secreted protein that promotes neural cell adhesion and neurite extension. *Cell* **69**: 95–110
- Kuhn R, Schwenk F, Aguet M, Rajewsky K (1995) Inducible gene targeting in mice. *Science* **269**: 1427–1429
- Liakka KA, Autio-Harmanen HI (1992) Distribution of the extracellular matrix proteins tenascin, fibronectin, and vitronectin in fetal, infant, and adult human spleens. *J Histochem Cytochem* **40**: 1203–1210
- Lindquist RL, Shakhbar G, Dudziak D, Wardemann H, Eisenreich T, Dustin ML, Nussenzweig MC (2004) Visualizing dendritic cell networks *in vivo*. *Nat Immunol* **5**: 1243–1250
- Lo CG, Lu TT, Cyster JG (2003) Integrin-dependence of lymphocyte entry into the splenic white pulp. *J Exp Med* **197**: 353–361
- Miller MJ, Safrina O, Parker I, Cahalan MD (2004) Imaging the single cell dynamics of CD4⁺ T cell activation by dendritic cells in lymph nodes. *J Exp Med* **200**: 847–856
- Miranti CK, Brugge JS (2002) Sensing the environment: a historical perspective on integrin signal transduction. *Nat Cell Biol* **4**: E83–E90
- Pasare C, Medzhitov R (2003) Toll pathway-dependent blockade of CD4⁺CD25⁺ T cell-mediated suppression by dendritic cells. *Science* **299**: 1033–1036
- Pasare C, Medzhitov R (2004) Toll-dependent control mechanisms of CD4T cell activation. *Immunity* **21**: 733–741
- Plow EF, Haas TA, Zhang L, Loftus J, Smith JW (2000) Ligand binding to integrins. *J Biol Chem* **275**: 21785–21788
- Pribila JT, Itano AA, Mueller KL, Shimizu Y (2004) The alpha 1 beta 1 and alpha E beta 7 integrins define a subset of dendritic cells in peripheral lymph nodes with unique adhesive and antigen uptake properties. *J Immunol* **172**: 282–291
- Price LS, Leng J, Schwartz MA, Bokoch GM (1998) Activation of Rac and Cdc42 by integrins mediates cell spreading. *Mol Biol Cell* **9**: 1863–1871

- Puig-Kroger A, Sanz-Rodriguez F, Longo N, Sanchez-Mateos P, Botella L, Teixeira J, Bernabeu C, Corbi AL (2000) Maturation-dependent expression and function of the CD49d integrin on monocyte-derived human dendritic cells. *J Immunol* **165**: 4338–4345
- Raghavan S, Bauer C, Mundschau G, Li Q, Fuchs E (2000) Conditional ablation of beta1 integrin in skin. Severe defects in epidermal proliferation, basement membrane formation, and hair follicle invagination. *J Cell Biol* **150**: 1149–1160
- Ren XD, Kiosses WB, Schwartz MA (1999) Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO J* **18**: 578–585
- Shortman K, Liu YJ (2002) Mouse and human dendritic cell subtypes. *Nat Rev Immunol* **2**: 151–161
- Shurin GV, Tourkova IL, Chatta GS, Schmidt G, Wei S, Djeu JY, Shurin MR (2005) Small rho GTPases regulate antigen presentation in dendritic cells. *J Immunol* **174**: 3394–3400
- Stoll S, Delon J, Brotz TM, Germain RN (2002) Dynamic imaging of T cell–dendritic cell interactions in lymph nodes. *Science* **296**: 1873–1876
- Swetman CA, Leverrier Y, Garg R, Gan CH, Ridley AJ, Katz DR, Chain BM (2002) Extension, retraction and contraction in the formation of a dendritic cell dendrite: distinct roles for Rho GTPases. *Eur J Immunol* **32**: 2074–2083
- Umekiya T, Takeichi M, Nose A (1997) M-spondin, a novel ECM protein highly homologous to vertebrate F-spondin, is localized at the muscle attachment sites in the *Drosophila* embryo. *Dev Biol* **186**: 165–176
- West MA, Prescott AR, Eskelinen EL, Ridley AJ, Watts C (2000) Rac is required for constitutive macropinocytosis by dendritic cells but does not control its downregulation. *Curr Biol* **10**: 839–848
- Wu X, Jiang N, Fang YF, Xu C, Mao D, Singh J, Fu YX, Molina H (2000) Impaired affinity maturation in Cr2^{−/−} mice is rescued by adjuvants without improvement in germinal center development. *J Immunol* **165**: 3119–3127
- Zhang M, Tang H, Guo Z, An H, Zhu X, Song W, Guo J, Huang X, Chen T, Wang J, Cao X (2004) Splenic stroma drives mature dendritic cells to differentiate into regulatory dendritic cells. *Nat Immunol* **5**: 1124–1133