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NIK Prevents the Development of Hypereosinophilic Syndrome-like Disease in Mice Independent of IKKα Activation

Hans Häcker,* Liying Chi,* Jerold E. Rehg,† and Vanessa Redecke*

Immune cell-mediated tissue injury is a common feature of different inflammatory diseases, yet the pathogenetic mechanisms and cell types involved vary significantly. Hypereosinophilic syndrome (HES) represents a group of inflammatory diseases that is characterized by increased numbers of pathogenic eosinophilic granulocytes in the peripheral blood and diverse organs. On the basis of clinical and laboratory findings, various forms of HES have been defined, yet the molecular mechanism and potential signaling pathways that drive eosinophil expansion remain largely unknown. In this study, we show that mice deficient of the serine/threonine-specific protein kinase NF-κB–inducing kinase (NIK) develop a HES-like disease, reflected by progressive blood and tissue eosinophilia, tissue injury, and premature death at around 25–30 wk of age. Similar to the lymphocytic form of HES, CD4+ T cells from NIK-deficient mice express increased levels of Th2-associated cytokines, and eosinophilia and survival of NIK-deficient mice could be prevented completely by genetic ablation of CD4+ T cells. Experiments based on bone marrow chimeric mice, however, demonstrated that inflammation in NIK-deficient mice depended on radiation-resistant tissues, implicating that NIK-deficient immune cells mediate inflammation in a nonautonomous manner. Surprisingly, disease development was independent of NIK’s known function as an IkB kinase α (IKKα) kinase, because mice carrying a mutation in the activation loop of IKKα, which is phosphorylated by NIK, did not develop inflammatory disease. Our data show that NIK activity in nonhematopoietic cells controls Th2 cell development and prevents eosinophil-driven inflammatory disease, most likely using a signaling pathway that operates independent of the known NIK substrate IKKα. The Journal of Immunology, 2012, 188: 4602–4610.

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Abbreviations used in this article: Aire, autoimmune regulator; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; HES, hypereosinophilic syndrome; IKKα, IkB kinase α; MBP, mannose-binding protein; NIK, NF-κB–inducing kinase; Treg, regulatory T cell; wt, wild-type.

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leads to the stabilization and activation of NIK, which phosphorylates and activates IkB kinase α (IKKα) (14, 30). Activation of IKKα in turn leads to the phosphorylation and limited proteolysis of NF-κB/p100, resulting in the release of the RELB-binding N-terminal p52 moiety. The heterodimer of p52 and RELB translocates to the nucleus and initiates gene transcription (31). Accordingly, Nik−/− and Nikaly/aly mice show a complete defect in p100 processing (12 and H. Häcker and V. Redecke, unpublished observations). Nikaly/aly mice were shown to develop a spontaneous inflammatory disease, characterized by lymphoctic infiltrations in various organs (32–34), which has been attributed to a failure of the alternative NF-κB pathway in the thymic stroma environment, resulting in combined defects in negative and positive selection of autoreactive and regulatory T cells (Tregs), respectively (22, 24). Despite the development of this spontaneous inflammatory condition, also referred to as “autoimmune disease,” Nikaly/aly mice are refractory to disease development in autoimmune disease models of arthritis, experimental autoimmune encephalomyelitis (EAE), and graft-versus-host disease (35–38), which in part may be explained by reduced production of IL-2 by T cells from NIK-deficient mice, as well as reduced generation of Th17 cells (22, 24, 36, 39). The resistance of NIK-deficient mice to disease development in autoimmune disease models, however, appeared to reflect a less well characterized function of NIK in dendritic cells (DCs) rather than a T cell-intrinsic defect (35). Nevertheless, adoptive transfer of CD4+ Nikaly/aly T cells into Rag2−/− mice (34) was found to be sufficient to induce inflammatory disease, suggesting that NIK-deficient CD4+ T cells exert a hitherto uncharacterized effector function that triggers inflammatory disease.

We sought to further study inflammatory disease development in Nik−/− mice and to determine the role of CD4+ T cells in disease progression. We found that inflammation in NIK-deficient mice, as reflected by increased numbers of leukocytes in peripheral blood and diverse tissues, was largely due to increased eosinophil numbers, thereby resembling a progressive HES-like disease, eventually resulting in tissue destruction and premature death. In this article, we characterize the eosinophilic inflammation in NIK-deficient mice, the phenotype of Nik−/− CD4+ T cells, and the contribution of radiation-resistant and hematopoietic tissue to inflammatory disease development. Furthermore, we investigate the role of NIK’s known downstream target (i.e., IKKα) for its contribution to disease. Together, our data suggest that NIK’s function in radiation-resistant tissue is essential for the prevention of T cell-mediated HES-like disease, which however does not depend on NIK’s known function as an IKKκ kinase.

Materials and Methods

Mice

Nik−/− mice (Map3k14tm1Rhib), generated by Yin and colleagues (26), were kindly provided by Amgen and backcrossed onto the C57BL/6 mouse background for nine generations. IKKα−/− mice (40) were a kind gift from Michael Karin (University of California at San Diego, La Jolla, CA). C57BL/6 mice and Rag2−/− mice were obtained from The Jackson Laboratory (Bar Harbor, ME). MHC class II−/− mice (B6.129-H2−B2m−/−) and SJL mice were purchased from The Jackson Laboratory (Tree Star).

CD4+ T cell isolation

Single-cell suspensions were prepared from the spleen by straining tissues through a 100-µm cell strainer (BD Biosciences). RBCs were lysed using ammonium-chloride-potassium lysis buffer (Lonza), and CD4+ T cells were isolated by positive selection with anti-CD4 microbeads (Miltenyi Biotec). Cells were cultured in RPMI 1640 (Invitrogen) supplemented with 10% (v/v) FCS (Hyclone), 50 mM 2-ME, and antibiotics (penicillin G (100 IU/ml) and streptomycin sulfate (100 IU/ml); Invitrogen).

Cytokine measurement

Purified CD4+ T cells were stimulated with plate-bound anti-CD3/anti-CD28 (10 µg/ml; eBioscience) for 72 h, and the concentrations of IL-5, IL-10, IFN-γ (BD Biosciences), IL-4, and IL-13 (eBioscience) were determined from the supernatant by ELISA according to the manufacturer’s instructions.

Histology and complete blood cell counts

Sixteen-week-old mice were perfused with 10% phosphate-buffered formalin and embedded in paraffin. Tissues were sectioned at 5 µm and stained with H&E, rat anti-mouse mannose-binding protein (MBP) polyclonal Ab (clone MF-147; Mayo Clinic, Rochester, MN), or goat anti-mouse CD3 (Santa Cruz Biotechnology) to detect eosinophil and T cell infiltrations respectively.

Peripheral blood was collected by retroorbital bleeding. Complete blood cell counts were measured using the ForCyte hematology system (Oxford Science). Differential blood counts were determined manually by a trained pathologist by microscopic examination of Wright’s stained peripheral blood smears. At least 100 WBCs were counted.

Flow cytometry analysis

Single-cell suspensions of the spleen were prepared and blocked with Abs against CD16/CD32 (eBioscience), followed by staining for cell surface expression of CD3 (145-2C11), CD4 (RM4-5), GR-1 (Ly6G, RB6-8C5), CD44 (IM7), CD62L (MAL-14) (eBioscience), CD8 (53-6.7), Siglec-F (E50-2440), and CCR3 (CD193) (83103) (BD Biosciences). Flow cytometry data were acquired on a FACS-Calibur flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (Tree Star).

Generation of bone marrow chimera mice

Bone marrow chimeras were generated by reconstituting irradiated (950 Gy) 6- to 10-wk-old CD45.1+ SJL or CD45.2+ recipient mice with 1 × 106 bone marrow cells from SJL or Nik−/− donor mice. Chimerism was verified by analysis of CD45.2 expression on peripheral blood cells by flow cytometry.

Statistical analysis

Data are expressed as the mean ± SEM and were compared using Student t tests. A p value <0.05 was considered significant.

Results

NIK-deficient mice develop progressive systemic eosinophilia

Mice lacking functional NIK were shown to develop an inflammatory multiorgan disease characterized by lymphocytic infiltrations in various organs, such as the liver, lung, pancreas, and salivary glands (32–34). In this study, we sought to further characterize the development of inflammatory disease in Nik−/− mice over time, including the cellular composition of the leukocytic organ infiltrations and the role of T cells in this process. We found that NIK-deficient mice develop a progressive disease characterized by thickening of the eyelids, fur loss, and skin inflammation around 11.4 ± 0.6 wk and resulting in premature death around 21.2 ± 5 wk (Fig. 1A, 1B). There was no significant difference in disease development between male and female mice (Fig. 1C).

Complete and differential blood counts showed a 1.9-fold increase in WBCs in Nik−/− mice; interestingly, this increase was due to elevated numbers of myeloid cells, in particular the number of monocytes and eosinophils, which were increased 4.5- and 7.5-fold, respectively (Fig. 1D). Eosinophils displayed a typical surface phenotype of GR-1+ Siglec-F+ cells with variable expression levels of CCR3, possibly reflecting different maturation stages (Fig. 1E) (41). No significant difference was detectable in the number of neutrophilic granulocytes or lymphocytes in the pe-
Peripheral blood. Histological examination revealed that virtually all organs were infiltrated with mononuclear cells. Consistent with results obtained from Nkx1.1−/− mice (33), these infiltrates contained CD3+ T cells (Fig. 1F, 1G); importantly, however, the infiltrates also contained eosinophilic granulocytes, as confirmed by MBP staining, which were dominating in all organs investigated (Fig. 1F, 1G).

Particularly strong infiltrations were seen in the lung (perivascular, interstitial, and alveolar), liver (portal), spleen, and skin, which were accompanied by apparent alterations of physiological tissue structure and integrity (Fig. 1F, 1G). Taken together, Nkx1.1−/− mice develop progressive blood and tissue eosinophilia accompanied by multiorgan damage and premature death.

**T cells in NIK-deficient mice exhibit a Th2-biased phenotype and a reduced CD4+/CD8+ ratio**

As shown above, Nkx1.1−/− mice develop infiltrations of T cells and eosinophils in multiple organs. Given that Th2 cell-derived IL-5 is a major maturation and differentiation factor for eosinophils, we next evaluated the cytokine profiles of CD4+ T cells isolated from the spleens of Nkx1.1−/− and littermate control mice in response to anti-CD3/anti-CD28 stimulation. We found significantly increased levels of IL-5, IL-4, IL-13, and IL-10 and reduced levels of IFN-γ in the supernatants of Nkx1.1−/− CD4+ T cells (Fig. 2A). This apparent Th2 bias of Nkx1.1−/− T cells was already detectable in 2-wk-old mice, thereby preceding symptoms of the described inflammatory disease, which becomes apparent at ∼11 wk of age (Figs. 1B, 2B).

Overall, progressive blood and tissue eosinophilia in the presence of a Th2-biased T cell phenotype in Nkx1.1−/− mice resembled the human lymphocytic form of HES. Because this form of HES also has been found to be associated with a decrease in the CD4+/CD8+ ratio in several HES patients (7), we next determined the percentage of CD3+CD4+ and CD3+CD8+ T cells in the spleen by flow cytometry. Indeed, we found a reduced CD4+/CD8+ T cell ratio in NIK-deficient mice, whereas the overall percentage of
CD4+ T cells are critically involved in the development of inflammatory disease in Nik−/− mice.

To further determine whether spontaneous progressive eosinophilia and Th2 bias in Nik−/− mice are results of an intrinsic defect of Nik−/− eosinophils or due to the described changes in T cell biology, we crossed Nik−/− mice into T cell-deficient mice and examined survival, development of eosinophilia, and multiorgan inflammation. Interbreeding Nik−/− mice into both Rag1−/− mice, which lack T and B cells, and MHC class II-deficient mice, which lack CD4+ T cells, completely protected the mice from disease development (Fig. 3). Although Nik−/− Rag1+/− and Nik−/− MhcI−/− mice succumbed to disease between 16 and 36 wk, Nik−/− Rag1−/− mice as well as Nik−/− MhcII−/− mice were protected completely from premature death (Fig. 3A, 3C). Accordingly, histological analyses of Nik−/− Rag1−/− mice and Nik−/− MhcII−/− mice showed no signs of inflammation or eosinophil infiltration in any organs, as shown for the lung and liver (Fig. 3B, 3D and data not shown). These results show that CD4+ T cells are involved critically in disease development in Nik−/− mice. The results also indicate that progressive eosinophilia is not a result of an intrinsic defect in Nik−/− eosinophils but rather a consequence of altered CD4+ T cell biology.

Th2 deviation and inflammatory disease in Nik−/− mice depend on nonhematopoietic cells

As shown by the experiments based on T cell-deficient mice, NIK-deficient eosinophils are not sufficient to mediate inflammatory disease but depend on NIK-deficient CD4+ T cells. To test whether inflammation is due to intrinsic defects of hematopoietic cells, including T cells, we generated chimeric mice by adoptive transfer of bone marrow from CD45.1+ SJL wt and CD45.1+ Nik−/− mice into lethally irradiated CD45.1+ SJL wt or CD45.1+ Nik−/− recipient mice and analyzed survival, T cell-dependent cytokine production, and eosinophil expansion. As shown in Fig. 4, all of the phenotypic changes associated with the inflammatory disease described in Nik−/− mice correlated with the NIK-deficient genotype of radiation-resistant, nonhematopoietic tissues but were

FIGURE 2. Th2-biased T cell phenotype and reduced CD4+/CD8+ T cell ratio in NIK-deficient mice. (A) Purified CD4+ T cells from 6- to 16-wk-old Nik−/+ and Nik−/− mice were left untreated (co) or stimulated with anti-CD3/anti-CD28 for 72 h, and cytokine levels were determined by ELISA. n = 9; data represent mean ± SEM; *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.0005. (B) Purified CD4+ T cells from 2-wk-old Nik−/+ and Nik−/− mice were stimulated with anti-CD3/anti-CD28 in triplicate wells and analyzed as described in (A). (C) Flow cytometry analysis of splenocytes from 6- to 12-wk-old Nik−/+ and Nik−/− mice. Total splenocytes (left panels), CD3+ gated cells (center panel), or CD3+CD4+ gated cells (right panel) are shown. n = 3; data represent mean ± SEM; CD8+ Nik−/+ versus CD8+ Nik−/−, p = 0.005; CD4+ Nik−/+ versus CD4+ Nik−/−, p = 0.002.
and survival of offspring was monitored for 40 wk. SJL NIK−/− mice were crossed to Rag1−/− mice, and survival of offspring was monitored for 40 wk. NIK−/− Rag−/− (n = 5) and NIK−/− Rag+/− (n = 10). (B) Histological analysis based on H&E and MBP staining of lung and liver of 16-wk-old NIK−/− Rag−/− and NIK−/− Rag+/− mice. Representative data obtained from six mice are shown. (C) NIK−/− mice were crossed to MhcII−/− mice, and survival of offspring was monitored for 40 wk. NIK−/− MhcII−/− (n = 10) and NIK−/− MhcII+/− (n = 10). (D) Histological analysis based on H&E and MBP staining of lung and liver of 16-wk-old NIK−/− MhcII−/− and NIK−/− MhcII+/− mice. Representative data obtained from three mice are shown.

FIGURE 3. Inflammation in NIK−/− mice depends on CD4+ T cells. (A) NIK−/− mice were crossed to Rag1−/− mice, and survival of offspring was monitored for 40 wk. NIK−/− Rag−/− (n = 5) and NIK−/− Rag+/− (n = 10). (B) Histological analysis based on H&E and MBP staining of lung and liver of 16-wk-old NIK−/− Rag−/− and NIK−/− Rag+/− mice. Representative data obtained from six mice are shown. (C) NIK−/− mice were crossed to MhcII−/− mice, and survival of offspring was monitored for 40 wk. NIK−/− MhcII−/− (n = 10) and NIK−/− MhcII+/− (n = 10). (D) Histological analysis based on H&E and MBP staining of lung and liver of 16-wk-old NIK−/− MhcII−/− and NIK−/− MhcII+/− mice. Representative data obtained from three mice are shown.

not affected by the genotype of bone marrow-derived cells. NIK−/− mice that were reconstituted with SJL wt or NIK-deficient bone marrow cells died prematurely ∼15 wk after transfer (Fig. 4A). In contrast, SJL wt mice that had received bone marrow from SJL wt or NIK−/− mice survived without any apparent disease symptoms. Survival correlated with the appearance of increased numbers of eosinophils in the peripheral blood and spleen of NIK−/− mice, irrespective of the genotype of bone marrow cells transferred (Fig. 4B, 4C). Moreover, SJL wt and NIK-deficient CD4+ T cells isolated from NIK−/− recipient mice produced very high levels of IL-5, whereas IFN-γ levels were reduced (Fig. 4D). In contrast, SJL wt and NIK-deficient CD4+ T cells isolated from SJL wt recipient mice produced much lower amounts of IL-5 but higher amounts of IFN-γ (Fig. 4D). Thus, the major characteristics of the described HES-like disease found in NIK−/− mice depend on radiation-resistant, nonhematopoietic cells, whereas T cells and eosinophils are required for disease development but are insufficient to trigger disease autonomously.

Disease in NIK−/− mice proceeds independent of its downstream target IKKα

We next wanted to test whether NIK’s inhibitory role on Th2 development was mediated by its known function as an IKKα kinase. IkkaAA/AA mice die perinatally due to defects in skeletal and epidermal development (42–44). We therefore used IkkaAA/AA knockin mice that carry point mutations in the IKKα activation loop (Ser179/Ser180), thereby disabling NIK-mediated IKKα activation (11, 40). Comparable to cells from IkkaAA/AA and NIK−/− mice, IkkaAA/AA cells exhibit defects in p100 processing; moreover, comparable to NIK−/− mice, IkkaAA/AA mice show defects in lymphoid organogenesis and B cell maturation and survival (11). Interestingly, IkkaAA/AA mice neither died prematurely nor did they develop any of the typical skin and eye lesions observed in NIK−/− mice (data not shown). This was not due to residual differences in the background of the different mouse strains used, because offspring of intercrossings of NIK−/− mice and IkkaAA/AA mice showed the same phenotype (Fig. 5A and data not shown). Although NIK−/− IkkaAA/AA mice died between 12 and 24 wk, no death was observed in the NIK−/− IkkaAA mice for >30 wk (Fig. 5A). A more detailed analysis of the peripheral blood in IkkaAA/AA mice showed some increase in leukocyte numbers, which were however mainly due to increased numbers of lymphocytes rather than granulocytes or monocytes (Fig. 5B). Eosinophil cell numbers in IkkaAA/AA mice were comparable to those of wt mice, in both peripheral blood and spleen, and no signs of eosinophilic organ infiltration were detected (Fig. 5B, 5C). We next analyzed the cytokine profile of CD4+ T cells from IkkaAA mice and their respective wt littermate controls. In contrast to the increased production of anti-CD3/anti-CD28-induced Th2 cytokines observed in NIK−/− CD4+ cells, IkkaAA CD4+ T cells did not show any increase in the release of Th2 cytokines (Fig. 5D). In fact, levels of both Th1- and Th2-associated cytokines (i.e., IL-5, IL-4, IL-13, IL-10, and IFN-γ) were reduced upon anti-CD3/anti-CD28 stimulation in comparison with those of their wt littermate con-
controls. Taken together, the data implicate that NIK controls Th2 differentiation and inflammatory disease independent of its known function through IKKα phosphorylation.

**Discussion**

In this study, we define NIK as an anti-inflammatory protein, whose function is essential for the regulation of Th2 development and protection from Th2-associated inflammatory disease. This disease shares several key characteristics of human HES, including increased numbers of eosinophils in the peripheral blood and eosinophilic infiltrations in virtually all organs including the skin, lung, liver, and spleen. Eosinophilic infiltrations were accompanied by progressive changes in organ architecture and fibrosis, most likely a consequence of toxic mediators produced by eosinophils, such as eosinophil peroxidase or MBP (45, 46). Two earlier histological studies described organ-specific eosinophilic infiltration in the stroma of the epididymis/vas deferens (47) and in the skin (48) in Nikaly/aly mice rather than revealing the general inflammation apparent in the Nik−/− mice described here. Whether these disparities reflect an incomplete defect of the signaling function of Nikaly/aly, which harbors a point mutation in the C-terminal part of NIK that contributes to IKKα binding (21, 49), or the more restricted scope of the mentioned studies is currently unclear.

Similar to the lymphocytic form of HES, Nik−/− CD4+ T cells produce significantly increased levels of Th2 cytokines, including IL-5, which controls survival, maturation, recruitment, expansion, and activation of eosinophils (Fig. 2A) (50, 51). Although IL-5 can be produced by other cell types such as basophils, mast cells, and NK cells, the main producer of IL-5 and initiators of eosinophil recruitment into tissues appear to be Th2 T cells, at least as far as investigated during allergic inflammation (52). A critical contribution of Th2 cells to the eosinophilia in Nik−/− mice is supported strongly by the observation that Nik−/− mice intercrossed into T cell-deficient mice (i.e., Rag1−/− mice and MhcII−/− mice) are resistant to eosinophilic inflammation and premature death (Fig. 3). These data also suggest that expansion of eosinophils is not a cell-autonomous function of NIK-deficient eosinophils. Interestingly, experiments based on bone marrow chimeras further show that all of the parameters of the inflammatory disease described (i.e., Th2 deviation, eosinophil expansion, and premature death) are triggered through radiation-resistant but not hematopoietic tissues. As such, inflammation observed in NIK-deficient mice is mediated by T cells in a nonautonomous fashion. An important role for peripheral T cells in the pathogenesis of inflammatory disease in Nik mutant mice, sometimes referred to as autoimmune disease, has been supported by several animal and clinical studies. Adoptive transfer of Nikaly/aly CD4+ cells into Rag2−/− mice was shown to result in severe mononuclear cell infiltrations and tissue destruction in various organs 4–10 wk after transfer (34). Also, HES patients have been treated successfully with agents that are known to suppress T cell activation (e.g., cyclosporine or 2-chlorodeoxyadenosine) (53–56), supporting the idea that T cells contribute to HES disease.

Even though Nik−/− and Nikaly/aly mice develop a T cell-dependent inflammatory disease, the adaptive immune responses to foreign Ag were found to be impaired in these mice (29), and both Nik−/− and Nikaly/aly mice are resistant to EAE and graft-versus-host disease (35–37). Disease resistance was attributed to a failure to induce Th17 cells and also to a reduced capacity to upregulate IL-2 upon TCR activation, referred to as T cell anergy. T cell anergy was suggested to be a result of a defect in T cell development due to altered function of NIK-deficient thymic DCs (35). Consistent with these reports, we found that Nik-deficient CD4+ T cells produce lower levels of IL-2 and IFN-γ than wt cells upon anti-CD3/anti-CD28 stimulation (Fig. 2A and data not shown). However, we also found that reduced production of these...
cytokines was accompanied by exaggerated production of Th2 cytokines, strongly suggesting classic Th2 deviation rather than T cell anergy as the primary phenotype of Nik−/−/CD4+ T cells. The role of NIK in the prevention of Th2 responses is supported further by the fact that gene vaccination using NIK as an adjuvant induces Th1 immune responses (57).

Given the strong Th2 bias observed in Nik−/− mice, upon worm infections, one would assume a more efficient immune response, which is mediated primarily by Th2 cells, in NIK-deficient mice. However, Th2 responses also were found to be impaired in NIK−/− mice in a model of *Trichinella spiralis* infection (58). Although the described results again may reflect differences between Nik−/− and NIK−/− mice, another possibility is that more complex immune responses, as required during pathogen challenge, may depend on the integrity of various lymphoid organs, such as lymph nodes and Peyer’s patches, which are missing in NIK-deficient mice. As such, it is currently unclear whether impaired cellular immune responses to foreign Ag are due to an intrinsic defect in immune cells, including T cells, or merely a consequence of the described defects in lymphoid organ development.

Interestingly, the response of Nik−/− T cells to foreign Ag in the EAE model could be restored upon expression of NIK in DCs (35). Moreover, changes in DC biology in NIK−/− mice also have been reported by Tamura et al. (59), who found that the overall number of DCs, the surface expression of CD80, CD86, and MHC class II, and the ability to present Ag were reduced in cells from NIK−/− mice, suggesting that NIK deficiency in DCs leads to impaired adaptive immunity to foreign Ag. Although NIK expression in DCs seems to be important for the generation of adaptive immune responses to foreign Ag, NIK-expressing DCs seem not to be the cause for the development of the spontaneous multiorgan inflammation observed in Nik−/− mice (28; Fig. 4). Thymus transfer experiments showed that the engraftment of thymus from NIK−/− mice in nude mice was sufficient to induce leukocytic organ infiltration, possibly reflecting the inflammatory disease described here for Nik−/− mice (28). Those results suggest that thymic stroma cells, specifically medullary thymic epithelial cells, control the aberrant T cell biology rather than DCs. Although it may be possible in principle that DCs also were adoptively transferred as part of the organ, these thymic transfer experiments in combination with our results based on bone marrow chimeric mice strongly suggest nonhematopoietic cells, most likely thymic stroma cells, as the disease-initiating factor.

In the above-mentioned thymus transfer study, the multiorgan inflammation observed in Nik−/− mutant mice was interpreted to be a result of impaired tolerance induction and impaired generation of natural Tregs due to reduced expression of the autoimmune regulator (Aire), which controls thymic expression of tissue-restricted self Ags (28, 60). Although impaired tolerance induction and reduced levels of regulatory T cells are feasible explanations for the inflammatory phenotype observed in NIK mutant mice, there are several observations that should be noted. First, although Aire−/− mice also develop a generalized inflammatory, autoimmune disease with lymphocytic infiltrations (61, 62), T cells in these mice exhibit a prominent Th1 phenotype with...
enhanced secretion of IFN-γ (63). Second, we did observe a similar decline in the number of Tregs in Ikkα/AA/AA mutant mice (Supplemental Fig. 1), which however do not develop signs of inflammation (Fig. 4). As such, it is unlikely that impaired tolerance induction and impaired generation of natural Tregs due to reduced expression of Aire are the sole reasons for the observed Th2 deviation in NIK−/− mice. The data obtained from Ikkα/AA/AA mutant mice also support the idea that Treg numbers are regulated via a classic NIK–IKK pathway, whereas the observed HES-like disease in NIK-deficient mice is not (see below). Taking our data and described studies together, it seems possible that the inflammatory phenotype of NIK−/− mice is the result of a combination of the impaired deletion of self-reactive T cells in a NIK-deficient thymus stroma environment, the absence of sufficient priming by DCs toward Th1 and other Th fates, and the reduced numbers of Tregs, which together lead to the expansion of autoreactive, Th2-biased T cells and (consecutive) IL-5-driven eosinophils, which ultimately mediate a HES-like inflammatory disease.

Somewhat surprising, disease development in NIK−/− mice was independent of NIK’s classical function as an IKKα kinase, suggesting that NIK controls Th2 responses by mechanisms independent of the phosphorylation and activation of IKKα. Characterized functions of NIK in the regulation of lymphoid organogenesis, B cell development, and the generation of Th17 cells were found to depend on critical phosphorylation in the activation loop of IKKα, which is mutated in Ikkα/AA/AA mice (11, 64). Although Ikkα was shown to regulate effector functions independent of NIK (65), a signaling pathway allowing NIK to act independent of Ikkα has not been characterized. In contrast to NIK−/−/− mice, a recent study found that Ikkα/AA/AA mutant mice mount normal primary Ab responses (66), suggesting that NIK might act independent of Ikkα, at least in B cells. Although our data do not entirely rule out the possibility that Ikkα is required for NIK-dependent prevention of inflammation, it appears that the classic phosphorylation-dependent signaling cascade triggered through NIK-mediated IKKα phosphorylation is either not relevant or compensated by other mechanisms (i.e., an Ikkα-independent signaling pathway). More sophisticated mouse models (e.g., based on tissue-specific Ikkα knockout strains or biochemically identification of additional NIK substrates) will be required to address this question.

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Disclosures

The authors have no financial conflicts of interest.

References


Supplementary Figure 1

SUPPLEMENTARY FIGURE 1. *Nik*<sup>−/−</sup> and *Ikka*<sup>AA/AA</sup> mice show a reduction in T<sub>reg</sub>. Number of T<sub>reg</sub> (CD4<sup>+</sup>CD25<sup>+</sup> FOXP3<sup>+</sup>) were determined by flow cytometry in 16 week old *Nik*<sup>−/−</sup> and *Ikka*<sup>AA/AA</sup> mice and their respective littermate *wt* controls. n=3, results are shown as mean±SEM.