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T-cell-specific deletion of gp130 renders the highly susceptible IL-10-deficient mouse resistant to intestinal nematode infection

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Gp130 is the common receptor of the IL-6 family of cytokines and is involved in many biological processes, including acute phase response, inflammation and immune reactions. To investigate the role of gp130 under inflammatory conditions, T-cell-specific conditional gp130 mice were first bred to the IL-10-deficient background and were then infected with the gastrointestinal nematode *Trichuris muris*. While IL-10$^{-/-}$ mice were highly susceptible to *T. muris*, developed a mixed Th1/Th17 response and displayed severe inflammation of the caecum, infection of mice with an additional T-cell-specific deletion of gp130 signalling completely reversed the phenotype. These mice showed an accelerated worm expulsion that was associated with the rapid generation of a strong Th2 immune response and a significant increase in Foxp3-expressing Treg. Therefore, gp130 signalling in T cells regulates a switch between proinflammatory and pathogenic Th1/Th17 cells and regulatory Th2/Treg *in vivo*. Taken together, the data demonstrate that gp130 signalling in T cells is a positive regulator of inflammatory processes, favouring the Th1/Th17 axis.

Key words: Cytokine receptor · Helminthic infection · Inflammation Th1/Th2/Th17 cells Transgenic mice

Introduction

The common receptor gp130 is constitutively expressed on immune and non-immune cells and is used by all members of the IL-6 family, which comprises IL-6, IL-11, leukaemia inhibitory factor, oncostatin M and the recently characterized cytokine IL-27, among others [1, 2]. While gp130 acts as the obligate signalling subunit, each cytokine first binds to its specific receptor, which accounts for the difference in activities [3, 4]. The lethality of mice with classical disruptions of two gp130 alleles demonstrated that gp130 signalling is important for the development of the nervous and hematopoietic systems [5, 6]. Moreover, signals mediated by gp130 regulate multiple other biological functions, including acute phase response, immune reactions and inflammation [7, 8]. The receptor is also involved in sustaining the disease state in inflammatory bowel disease, rheumatoid arthritis (RA) or MS [9–11].

The Th pathway is composed of, at least, three distinct subsets: Th1 cells produce IFN-γ and are involved in the fight against intracellular pathogens. The Th2 immune response with T cells producing IL-4, IL-5 and IL-13 provides immunity to helminths [2]. Th17 cells produce cytokines like IL-17, IL-22 and mediate immunity to extracellular bacteria and fungi, but are also responsible for autoimmunity and inflammation [12, 13]. Development of naïve T cells to Th17 cells requires the presence of IL-6, TGF-β1 and the transcription factor RORγt [14–17].

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Moreover, signalling through gp130 and activation of the STAT3 pathway in T cells have been shown to be critical for the development of Th17 cells [18]. In contrast, IL-27 also uses gp130 as signalling subunit but this cytokine acts as an immunosuppressor or a Th1-inducer and inhibits the promotion of a Th17 immune response [19]. The regulation of the different Th subsets is done by Treg and their development and function are regulated by TGF-β1 and the transcription factor Foxp3 [20, 21]. Since TGF-β1 is a common factor for Treg and Th17 cells, it is possible that a switch from Th17 to Treg exists *in vivo* in the absence of IL-6 signalling in T cells.

The gastrointestinal helminth *Trichuris muris* is a good model to investigate Th-cell pathways. In common inbred mouse strains (BALB/c, C57BL/6), infection with *T. muris* is characterized by the development of a Th2 immune response, eventually leading to resistance around day 21 post infection (p.i.) [22]. Although the mechanisms of worm expulsion are not fully understood, effector mechanisms such as increased epithelial-cell turnover, goblet-cell hyperplasia and mucus production are all involved in worm expulsion [23]. These changes in the environment of the large intestine are driven by the Th2 cytokine IL-13 and to a lesser extent IL-4 and IL-9 [22, 24]. In contrast, mice that develop a Th1 response remain susceptible and infection proceeds to chronicity [25]. In this context, IL-10-deficient mice infected with *T. muris* mount a Th1 immune response and develop a severe inflammation of the large intestine, resulting in the death of the mice [26].

Due to the lethality of gp130 complete knockout mice, the role of gp130 within the immune system remains unclear. In this study, we used the *T. muris* infection model to investigate the potential contributions of IL-6 family members in T–cell-specific inflammatory processes. Infections were done on an IL-10-deficient background in mice where we conditionally ablated the gp130 gene in T lymphocytes. While IL-$10^{-/-}$ mice exhibited a severe inflammation and displayed a cytokine profile of Th1 and Th17 cells after infection with *T. muris*, the disruption of gp130 signalling in T cells abolished the inflammation and rendered these mice highly resistant to infection. Our data provide evidence that gp130 signalling in T cells is required for both inflammatory axes, Th1 and Th17, and can eventually lead to chronic inflammation.

Results

IL-$10^{-/-}$ mice infected with *T. muris* show Th1 and Th17 cytokine profiles

In order to get significant inflammatory conditions to investigate the role of gp130 signalling in T cells, we considered using IL-$10^{-/-}$ mice, which have deregulated immune responses, develop spontaneous colitis and are highly susceptible to many infections [27, 28]. However, IL-$10^{-/-}$ mice in our animal facility do not develop colitis (M.C.G. and W.M., unpublished observations). Therefore, we used the gastrointestinal nematode *T. muris* to induce severe inflammation of the large intestine at day 21 p.i. and the development of a Th1 immune response in IL-$10^{-/-}$ animals [26]. In preliminary experiments, we found elevated Th1 cytokine levels after *in vitro* restimulation of the MLN cells with *T. muris* excretory-secretory (E/S) antigens at day 21 p.i. (Fig. 1A). Antigen-specific IL-12, IFN-γ and TNF-α were significantly upregulated, when compared with WT mice (Fig. 1A). Interestingly, Th17-related cytokines such as IL-17, IL-6 and GM-CSF [12, 29], were also significantly higher in IL-$10^{-/-}$ mice, when compared with WT control animals, indicating that IL-$10^{-/-}$ mice not only develop a Th1 immune response, but also an antigen-specific Th17 response after *T. muris* infection (Fig. 1A). As indicated by the high levels of proinflammatory cytokines and by published data [26], IL-$10^{-/-}$ mice developed a severe inflammation of the large intestine and 50% of the animals succumbed to infection from day 24 p.i. (Fig. 1B and data not shown). Inflammation was characterized by transmural infiltration of large numbers of granulocytes, severe oedema and extensive ulceration leading to disruption of the epithelial barrier (Fig. 1B). Histological scoring of caecal sections confirmed that the degree of inflammation was significantly higher at day 21 p.i. in IL-$10^{-/-}$ mice, when compared with WT animals (Fig. 1C). Taken together, these data indicate that infection of mice on the IL-10-deficient background with *T. muris* is a good model to investigate the role of gp130 in T cells under inflammatory conditions.

IL-17 is produced by non-T cells in IL-$10^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} at day 14 p.i.

Most of the resistant mouse strains expel worms around day 21 p.i., while IL-$10^{-/-}$ mice start to die around that time. Therefore, we sought to investigate the kinetics of the development of a protective or susceptible phenotype by first analysing the cytokine responses for the different T-cell subsets at day 14 p.i. At that time point, levels of IL-17 and IL-22 were surprisingly upregulated in IL-$10^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+}, as compared with IL-$10^{-/-}$ or WT mice (Fig. 2A). Analysis at the single-cell level using flow cytometry unveiled that the source of IL-17 was from non-T cells: IL-17 expression was significantly reduced in cultures of CD4^{+} T cells from IL-$10^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice as compared with cultures from IL-$10^{-/-}$ or WT (Fig. 2B). The low percentage of CD4^{+}IL-17^{+} cells was in agreement with the efficiency of gp130 deletion in CD4^{+} cells as revealed when analysed by FACS analysis (data not shown). These data indicate that gp130 signalling in T cells is essential for the development of Th17 cells. In contrast, IL-17 expression was upregulated in the CD4^{-} population in the cultures from IL-$10^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice (Fig. 2B). Known sources for IL-17 include γδ T cells, NK cells, NKT cells, neutrophils and eosinophils [30]. Here, the non-T-cell source of IL-17 is probably the neutrophil population, which is one of the primary cell types to be recruited after infection [31]. Since IL-6 and TGF-β1 have been shown to be the essential factors for the generation of the Th17 cell subset, a preferential differentiation of naïve T cells to Treg has been anticipated in the absence of IL-6. Using our *in vivo* infection model, we wanted to determine whether the lack of IL-6-dependent gp130 signalling in T cells would lead to an increase in

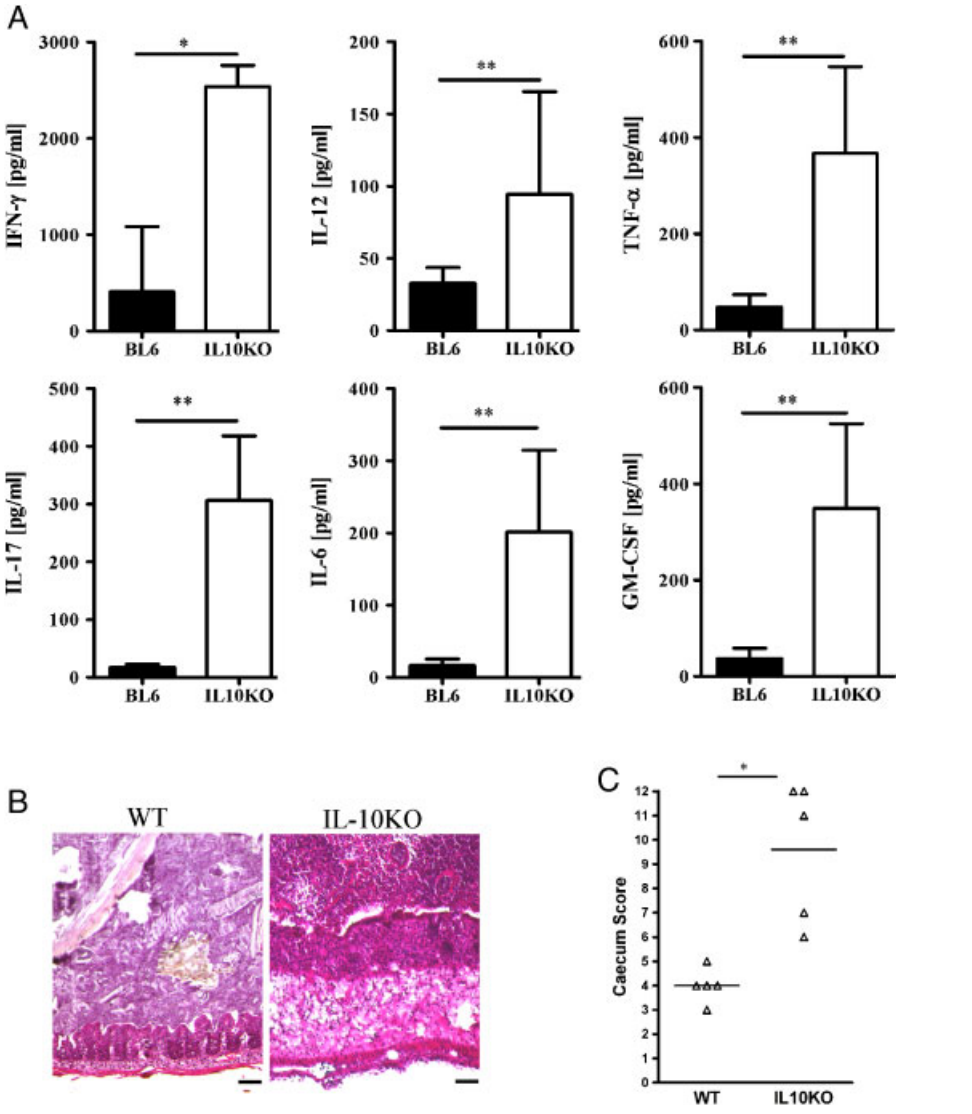

Figure 1. Th1 and Th17 cytokines are upregulated in IL-10-deficient mice following infection with *T. muris*. IL-10-deficient (IL-10KO) and WT mice (BL6) were infected with ~200 embryonated *T. muris* eggs. MLN cells were isolated at day 21 p.i. and cultured with parasite E/S antigen for 48 h. (A) Cytokine concentrations in supernatants were determined by Luminex technology. Data show mean and SD from five individual mice *per* group. (B) H&E staining of caeca from IL-10 and WT mice at day 21 p.i. Scale bar shows 100 μm. (C) Sections from B were blindly scored 0–3 for severity of inflammation, area involved, ulceration and presence of oedema. Data are representative of three independent experiments. *$p<0.05$, **$p<0.01$, Student's *t*-test.

Treg numbers *in vivo* after infection. FACS analysis of $CD4^+$ $CD25^+Foxp3^+$ Treg at day 14 p.i. showed a significant increase in their percentage only in cultures from IL-$10^{-/-}$gp$130^{fl/fl}$ CD4-Cre^+ (Fig. 2C). Taken together, these data suggest that a non-T-cell source of IL-17 is responsible for increased IL-17 production at day 14 p.i. and that the deletion of gp130 signalling in T cells induces a switch from Th17 to Treg *in vivo*.

IL-$10^{-/-}$gp$130^{fl/fl}$ CD4-Cre^+ mice develop an early and strong Th2 response

In addition to an increase in IL-17, Th1-associated cytokines could also be detected in supernatants from IL-$10^{-/-}$gp$130^{fl/fl}$ CD4-Cre^+ mice (Fig. 3A). IFN-γ, IL-12 and TNF-α were upregulated when compared with IL-$10^{-/-}$gp$130^{fl/fl}$ or WT mice (Fig. 3A). Similar to what we found for the source of IL-17, IFN-γ production was also from non-T-cell origin (data not shown), suggesting that the deletion of gp130 signalling in T cells leads to the abrogation of Th1 and Th17 cytokines by $CD4^+$ T cells but not by innate cells. Although an increase in proinflammatory cytokines was shown at day 14 p.i., their levels were much lower than Th2 cytokine levels, indicating that a Th2 immune response is the major response at that time point (Fig. 3B). Indeed, very high levels of IL-13, IL-4 and IL-5 were found in the restimulated cultures from IL-$10^{-/-}$gp$130^{fl/fl}$ CD4-Cre^+ mice, while none of these cytokines could be found in IL-$10^{-/-}$gp$130^{fl/fl}$ mice. The magnitude of the Th2 immune response on CD4-Cre^+ animals

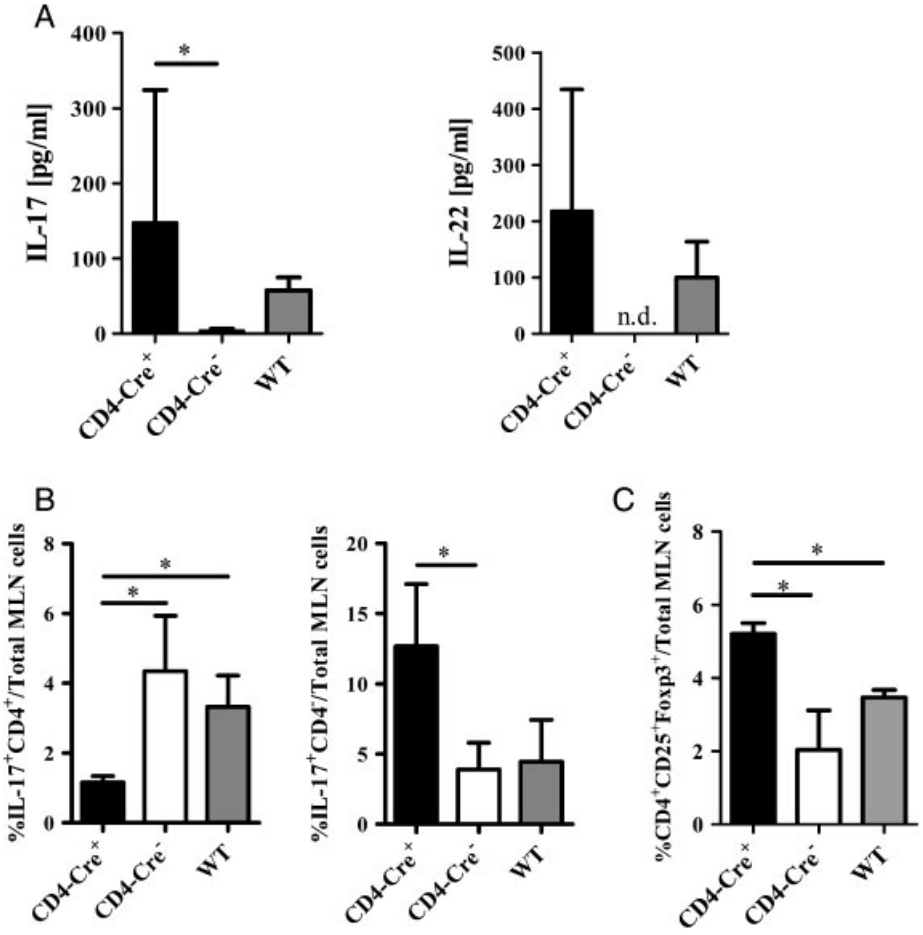

Figure 2. T-cell-specific deletion of gp130 in IL-$10^{-/-}$ mice leads to a transient increase in the production of Th17 cytokines and increased proportion of Treg at day 14 p.i. with *T. muris*. IL-$10^{-/-}$gp$130^{fl/fl}$ CD4-Cre^{+} (black bars), CD4-Cre^{-} (white) and WT (gray) mice were infected with ~200 embryonated *T. muris* eggs. MLN cells were isolated at day 14 p.i. and cultured with parasite E/S antigen for 48 h. (A) IL-17 and IL-22 concentration in the supernatant were determined by Luminex technology. At day 14 p.i., MLN cells were isolated and cultured with parasite antigen for 24 h; cells were then stained for CD4 and IL-17 (B) or for CD4, CD25 and Foxp3 (C). Data show mean and SD from five individual mice *per* group. Data are representative of three independent experiments. *$p<0.05$, Student's *t*-test.

showed at least a twofold increase when compared with WT mice (Fig. 3B). Therefore, we tested whether the different mouse strains were able to expel their worm burden by counting larvae at different time points. At day 11 p.i., homogeneous establishment of the infection within the different groups was assessed by counting larvae and was found to be similar for all mouse strains analysed (data not shown). In agreement with the very high Th2 levels found in the supernatants, IL-$10^{-/-}$gp$130^{fl/fl}$ CD4-Cre^{+} mice had already expelled half of their larvae by day 14 p.i. and had completed this process by day 21 p.i. (Fig. 3C). As expected, WT mice expelled worms after day 21 p.i., while IL-$10^{-/-}$gp$130^{fl/fl}$ mice remained susceptible and some of them eventually died of a severe inflammation of the large intestine from day 24 p.i. (Fig. 3C and data not shown). Taken together, these data indicate that the abrogation of gp130 signalling in T cells leads to the development of high levels of Th2 cytokines and Treg as well as a concurrent but transient increase in pro-inflammatory cytokines.

Th17 and Th1 responses are abrogated in IL-$10^{-/-}$gp$130^{fl/fl}$ CD4-Cre^{+} animals

Next, we analysed the cytokine response of the different Th subsets at day 21 p.i. Th17 cytokines, IL-17 and IL-22 were highly upregulated only in supernatants from infected IL-$10^{-/-}$gp$130^{fl/fl}$ mice (Fig. 4A). In contrast, FACS analysis revealed a strong decrease in IL-17 expression in the $CD4^{+}$ and the $CD4^{-}$ populations in cultures from IL-$10^{-/-}$gp$130^{fl/fl}$ CD4-Cre^{+} or WT, but not from IL-$10^{-/-}$gp$130^{fl/fl}$ animals (Fig. 4B and data not shown). Similar to what was observed for day 14 p.i., Treg percentages were again significantly higher in supernatants from IL-$10^{-/-}$gp$130^{fl/fl}$ CD4-Cre^{+} mice, demonstrating that the switch from Th17 cells to Treg *in vivo* in the absence of IL-6/gp130 signalling in T cells is not a transient process (Fig. 4B). As expected by the loss of IL-10, a population of $CD4^{+}$ IFN-γ^{+} cells was upregulated in cultures from IL-$10^{-/-}$gp$130^{fl/fl}$ mice, but not in resistant CD4-Cre^{+} or WT animals (Fig. 4B),

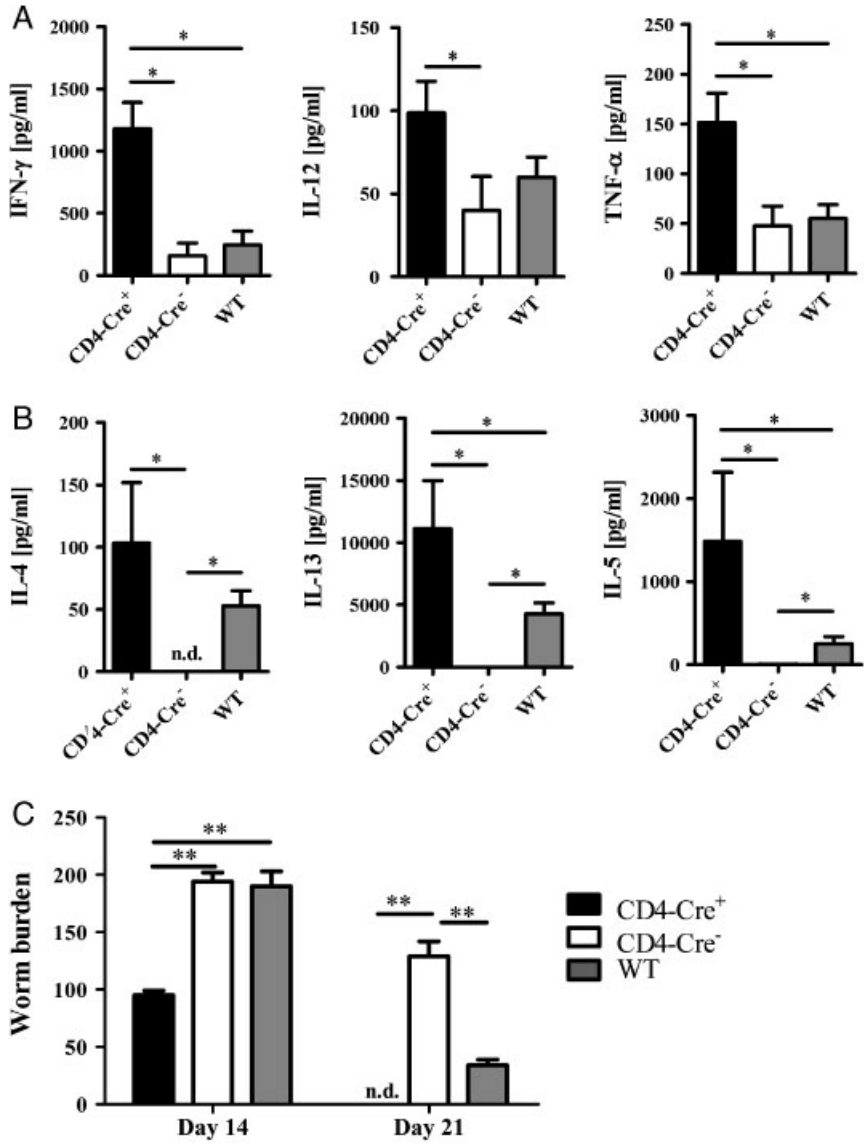

Figure 3. Infection with *T. muris* leads to a transient increase in Th1 cytokines at day 14 p.i.; however, the Th2 response is dominant, eventually leading to an accelerated larvae expulsion in IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice. IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} (black bars) and CD4-Cre^{-} (white) and WT (gray) mice were infected with ~200 embryonated *T. muris* eggs. MLN cells were isolated at day 14 p.i. and cultured with parasite E/S antigen for 48 h; concentrations of Th1 (A) and Th2 (B) cytokines in the supernatant were determined by Luminex technology. Detection limits are 5 pg/mL for IL-4 and 20 pg/mL for IL-13. n.d. not detected. Caecal worm burdens were assessed at day 14 p.i. or at day 21 p.i. (C) Data show mean and SD from five individual mice *per* group. Data are representative of three (B) or four (A and C) independent experiments. *p<0.05, **p<0.01, Student's t-test.

indicating that IL-10 is a negative regulator of the Th1 immune response.

In agreement with the course of worm expulsion, Th1 cytokines were downregulated in restimulated cultures from WT and IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice, but high levels of IL-12, IFN-γ and TNF-α were obtained in supernatants from IL-10$^{-/-}$gp130$^{fl/fl}$ mice (Fig. 4C). Although mice have already expelled their worm burden at day 21 p.i., Th2 cytokines were still present in supernatants from IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice at that time point; however, a threefold reduction was noted between day 14 and day 21 p.i. (Fig. 4D and 3B). Since WT mice are in the process of worm expulsion, higher levels of IL-13 were found in their supernatants when compared with CD4-Cre^{+} mice (Fig. 4D).

Different effector responses lead to resistance or susceptibility at day 14 p.i.

Since the type of response was very different in resistant WT and IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} as compared with IL-10$^{-/-}$gp130$^{fl/fl}$ mice, histological analysis was conducted at days 14 and 21 p.i. At day 14, IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice were in the process of expulsion and showed upregulation of proinflammatory

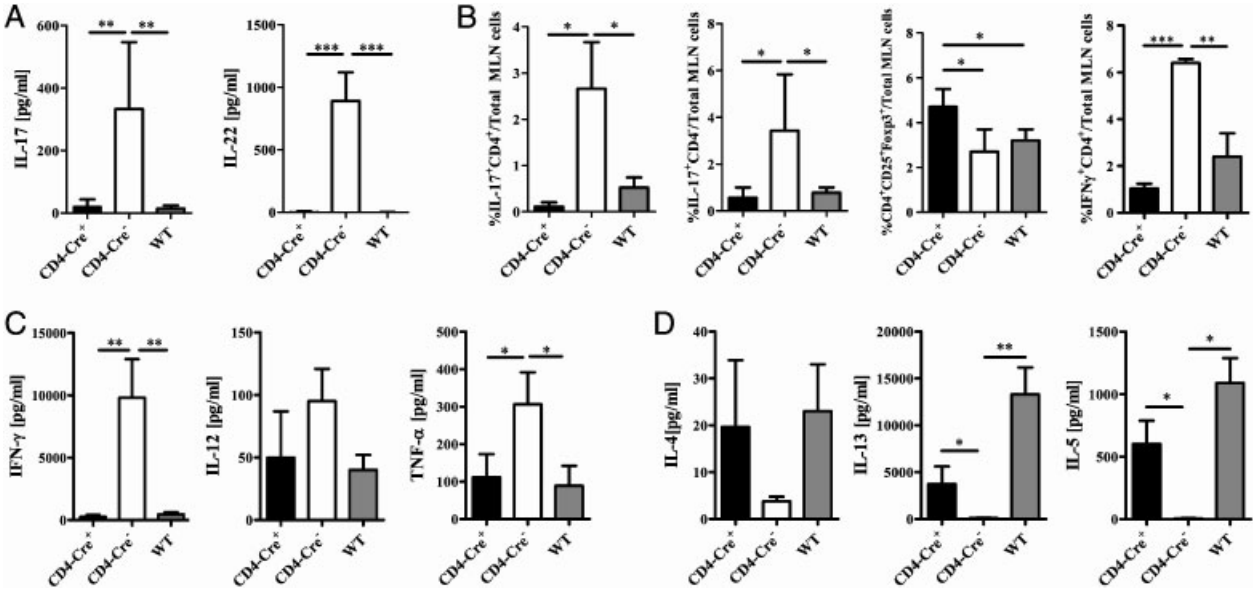

Figure 4. Deletion of gp130 signalling in T cells leads to the abrogation of the proinflammatory Th1 and Th17 immune responses at day 21 after infection with *T. muris*, with a concomitant increase in Th2. IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} (black bars) and CD4-Cre^{-} (white) and WT (gray) mice were infected with ~200 embryonated *T. muris* eggs. MLN cells were isolated at day 21 p.i and cultured with parasite E/S antigen for 48 h. (A) IL-17 and IL-22 concentration in the supernatant were determined by Luminex technology. For FACS analysis, MLN cells were cultured with E/S antigen for 24 h; cells were then stained for CD4, CD25, IL-17 and Foxp3 (B). For Th1 (C) and Th2 (D) cytokines, the same protocol as for (A) was applied. Data show mean and SD from five individual mice *per* group. Data are representative of three (B) or four (A, C, and D) independent experiments. *p<0.05, **p<0.01, ***p<0.001, Student's *t*-test.

cytokines as well as Th2 and Treg. In agreement with the worm burden and the cytokine data, IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice had a higher degree of inflammation at that time, when compared with IL-10$^{-/-}$gp130$^{fl/fl}$ mice, which showed only a mild diffuse enteritis (Fig. 5A and B). A trend towards an enhanced number of granulocytes could be observed in IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} animals, whereas lymphocytes appeared to be more prominent in IL-10$^{-/-}$gp130$^{fl/fl}$ mice (Fig. 5A). These data indicate that the upregulation of proinflammatory cytokines in resistant IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice leads to an influx of innate cells and an increase in the level of inflammation, positively resulting in worm expulsion. Moreover, the finding that granulocytes are the major population in IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice at day 14 p.i. is in agreement with the upregulation of IL-17 and IFN-γ by a non-T-cell source.

At day 21 p.i., histological scoring of caecal sections from IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} and WT mice exhibited almost no inflammation in comparison to IL-10$^{-/-}$gp130$^{fl/fl}$ animals, which had the highest score due to severe transmural invasion of inflammatory cells, extensive oedema and large ulcerations (Fig. 5A and B). Caeca from IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice showed only mild infiltration of inflammatory cells, no ulceration and oedema, and no disruption of the epithelial barrier (Fig. 5A). Taken together, the data indicate that gp130 signalling in T cells sustains the inflammation in an IL-10-deficient environment.

Worm expulsion has been associated with the Th2-cytokine IL-13, which induces effector mechanisms such as enhanced epithelial cell turnover and mucus production by goblet cells [23, 32]. Consistent with the upregulation of IL-13 after restimulation of MLN cells from IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} animals, the presence of an epithelial hyperplasia in the caecum was noted already at day 14 p.i. (Fig. 5A), together with a significant increase in the number of goblet cells as compared with WT or with IL-10$^{-/-}$gp130$^{fl/fl}$ animals (Fig. 5C). While IL-10$^{-/-}$gp130$^{fl/fl}$ mice exhibited a significant loss of goblet cells at day 21 p.i., the numbers of goblet cells found in the caeca of IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice were higher than those found in WT mice; however, the difference was not significant (Fig. 5C). Therefore, intestinal hyperplasia and increase in goblet cell numbers under the control of IL-13 are responsible for worm expulsion, either around day 14 p.i. in IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice or around day 21 p.i. in WT, while the loss of these cells could contribute to the intestinal inflammation seen in IL-10$^{-/-}$gp130$^{fl/fl}$ mice.

Taken together, the data demonstrate that the specific deletion of gp130 in T cells abrogates inflammation in a highly susceptible mouse strain, by suppressing the proinflammatory Th1 and Th17 responses, while allowing the generation of Treg and Th2 cells that are responsible for enhanced larvae clearance.

Discussion

In the present study, the role of T-cell-specific gp130 signalling in mice was investigated in the context of inflammation. In order to investigate the role of gp130 signalling in T cells and since IL-10-deficient mice did not develop colitis in our facility, we used the gastrointestinal helminth *T. muris* to induce inflammatory

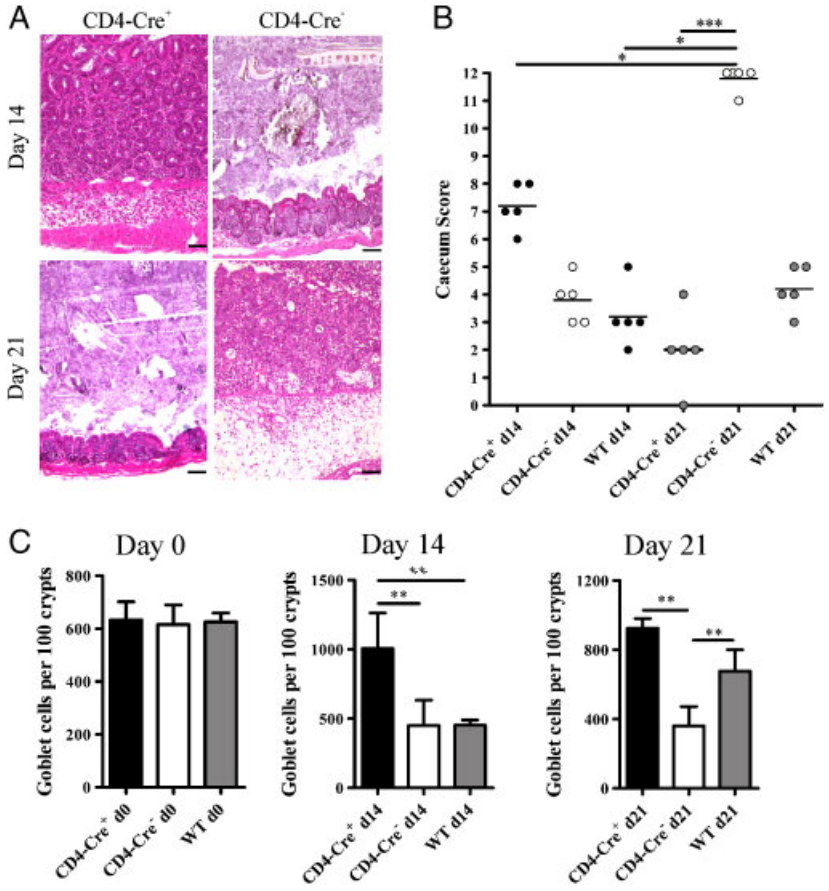

Figure 5. Inflammation and goblet cell numbers are differentially regulated in IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+}, CD4-Cre^{-} and WT mice during infection with *T. muris*. IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} (black dots), CD4-Cre^{-} (white) and WT (gray) mice were infected with ~200 embryonated *T. muris* eggs. (A) H&E staining of caeca from IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} and IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{-} mice at day 14 p.i. and day 21 p.i. (B) Sections from A were blindly scored 0–3 for severity of inflammation, area involved, ulceration and presence of oedema. Scale bar is 100 μm. (C) Goblet cell numbers from IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} (black bars), CD4-Cre^{-} (white) and WT mice (gray) were counted using periodic acid Schiff-stained caecal slides from uninfected animals (day 0) or *T. muris*-infected animals at day 14 p.i. and day 21 p.i. Data show mean and SD from five individual mice *per* group. Data are representative of three (B) or four (C) independent experiments. *p<0.05, **p<0.01, ***p<0.001, Kruskal–Wallis test with Dunns post test (B) and Student's *t*-test (C).

conditions. Common inbred strains generate a Th2 immune response and expel *T. muris* around 21–25 day p.i. In contrast, IL-10-deficient mice develop a Th1 immune response, characterized by high levels of IFN-γ, TNF-α and IL-12 after restimulation of the MLN cells with antigen at day 21 p.i. In addition to Th1 cytokines, high levels of Th17 cytokines were also found in the supernatants. Our results indicate that IL-10-deficient animals not only develop a Th1 immune response after infection with *T. muris*, but Th17 cytokines are upregulated, including IL-17 and IL-22. Moreover, these mice develop a severe inflammation of the large intestine and eventually die from a break in barrier function. Therefore, our model on the IL-10-deficient background is suitable for the analysis of gp130 signalling in T cells during inflammation.

In sharp contrast, Th1 and Th17 responses were completely abrogated at day 21 p.i. in IL-10$^{-/-}$ mice with an additional deletion of gp130 signalling in T cells, since no Th 1 and Th 17 cytokines could be found in the supernatants of cells from IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice. Moreover, the numbers of IFN-γ^{+} or IL-17^{+} cells were significantly lower in the MLN of IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice, when compared with IL-10$^{-/-}$gp130$^{fl/fl}$ animals. The level of inflammation was significantly reduced in IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice, demonstrating that the disruption of gp130 signalling in T cells leads to a strong decrease in the production of pro-inflammatory Th1 and Th17 cytokines and an abrogation in the recruitment of inflammatory cells. In this context, IL-6, through trans-signalling using sIL-6R, was shown to control the recruitment of neutrophils after acute inflammation [33–35]. Taken together, our data implicate a role of gp130 signalling in T cells for sustaining inflammation and perpetuating its chronicity.

Restimulated cells from IL-10$^{-/-}$gp130$^{fl/fl}$ CD4-Cre^{+} mice infected with *T. muris* displayed a Th2 profile, with significantly higher levels of the Th2 cytokines IL-4, IL-5 and IL-13. A similar phenotype has been shown when IL-27Rα$^{-/-}$ (WSX-1$^{-/-}$) mice were infected with *T. muris* [36]. These mice developed a strong overproliferative Th2 response, which led to accelerated worm expulsion before day 14 p.i [36]. Recent work has highlighted a role for IL-27 in the suppression of Th2 development of naïve

T cells and Th2 cytokine production by polarized Th2 cells by upregulating the expression of the Th1 transcription factor T-bet and simultaneously downregulating GATA3, essential for the Th2 response [37, 38]. As the effects of IL-27/gp130 signalling occur earlier in the infection process and are more direct than the responses driven by the lack of IL-6 and IL-10, the phenotype observed here masks the deleterious effects of uncontrolled IFN-γ production by Th1 cells that were shown to be regulated by an autocrine production of IL-10 [39, 40]. Even though this hypothesis was demonstrated using Th1 models of infection, we show here that IL-$10^{-/-}$ mice harbour a high percentage of $CD4^+$ IFN-γ^+ T cells at day 21 p.i., suggesting that the abrogation of the IL-10 negative feedback loop leads to an increase in pathogenic Th1 cells (Fig. 4B). In addition, levels of IL-17 and IL-17^+ cells were significantly lower in restimulated cultures from IL-$10^{-/-}$ $gp130^{fl/fl}$ CD4-Cre^+ mice. However, it is not known whether the downregulation of the Th17 response is also due to IL-27, since IL-27 was identified as an inhibitor of Th17-cell development [19]. Therefore, we conclude that the promotion of a strong and efficient Th2 response *in vivo* in IL-$10^{-/-}gp130^{fl/fl}$ CD4-Cre^+ mice most likely represents effects from IL-27-dependent gp130 signalling in T cells.

Addition of IL-6 in the absence of TGF-β converts $CD4^+CD25^+$ $Foxp3^+$ T cells to Th17 cells *in vitro*, and *trans*-signalling by IL-6 was recently shown to inhibit the *de novo* induction of Foxp3 expression in adaptive Treg leading to a loss of suppressive function [41–43]. Therefore, we investigated whether the decrease in Th17 cells seen in mice lacking gp130 signalling in T cells was accompanied by an augmentation of Treg numbers *in vivo*. Indeed, IL-$10^{-/-}gp130^{fl/fl}$ CD4-Cre^+ mice infected with *T. muris* showed a significantly higher percentage of $CD4^+CD25^+$ $Foxp3^+$ Treg at days 14 and 21 p.i., when compared with IL-$10^{-/-}gp130^{fl/fl}$ or WT mice, indicating that gp130 signalling in T cells is important in mediating the shift from Treg to Th17 cells. Recently, Korn and colleagues demonstrated that IL-6 signalling inhibits the conversion from conventional T cells into Foxp3-expressing Treg *in vivo* using a model of EAE [44]. Our results corroborate this finding and provide additional evidence that the disruption of the IL-6/gp130 signalling pathway in T cells leads to a preferential activation of Treg *in vivo* and controls the switch between Treg and Th17 cells.

Because of the rapid worm expulsion in IL-$10^{-/-}gp130^{fl/fl}$ CD4-Cre^+ mice, the development of antigen-specific responses was assessed at day 14 p.i. Restimulated MLN cells from these mice exhibited already at this stage a polarized Th2 profile with significantly higher levels of the Th2 cytokines IL-4, IL-5 and IL-13, when compared with the respective profile from IL-$10^{-/-}gp130^{fl/fl}$ mice. The cytokine production from IL-$10^{-/-}gp130^{fl/fl}$ CD4-Cre^+ mice at day 14 p.i. was even three times higher than the production at day 21 p.i. Nevertheless, a simultaneous increase in the expression levels of IFN-γ and IL-17 was monitored in restimulated MLN cultures from these mice. This effect was only transient at day 14 p.i., since these cytokines were then downregulated at day 21 p.i. Moreover, these cytokines were shown to be from non-T-cell sources (Fig. 2B and 4B), and most probably produced by innate cells such as neutrophils. Indeed, an increase in the number of granulocytes was found by histological analysis of caeca from IL-$10^{-/-}gp130^{fl/fl}$ CD4-Cre^+ mice and IL-6 was found to control the resolution of innate immunity and is one of the factors driving the transition to adaptive immune responses [11].

High levels of IL-13 have been associated with changes in the composition of the large intestine. Goblet cell hyperplasia, increase in mucus production, and enhanced epithelial cell turnover was shown to be controlled by IL-13 [23, 45]. Histological analysis of caeca from IL-$10^{-/-}gp130^{fl/fl}$ CD4-Cre^+ mice at day 14 p.i. revealed exaggerated goblet cell and epithelial cell hyperplasia. Again, this phenotype mimics the changes observed in WSX-$1^{-/-}$ mice [36]. Moreover, *T. muris* infection of IL-$10^{-/-}$ $gp130^{fl/fl}$ CD4-Cre^+ mice caused an influx of macrophages and neutrophils at day 14 p.i., which was in agreement with the augmented inflammation seen in the caecum and with the production of proinflammatory cytokines.

In conclusion, the deletion of gp130 signalling in T cells in mice favours anti-inflammatory and regulatory pathways. Since high levels of IL-6 and IL-27 have been found in patients with Crohn's disease [46], drugs inhibiting the IL-6/IL-27/gp130 pathway specifically in T cells could be of great interest as therapeutics against chronic inflammation. This particular blockade could provide a new and safe way for the concomitant generation of Th2 and Treg. In this context, blocking of the IL-6-driven inflammatory axis was already shown to be successful for the treatment of Crohn's disease in humans [47].

Materials and methods

Animals

Animals were bred in-house at the Helmholtz Centre for Infection Research under specific pathogen-free conditions and all experiments were performed in accordance with federal guidelines and institutional policies. All animals (IL-$10^{-/-}$ [27], RAG2$\gamma_c^{-/-}$ [48], $gp130^{fl/fl}$ [5] and CD4-Cre [49] were of a C57BL/6J background. Conditional $gp130^{fl/fl}$ mice were bred to IL-$10^{-/-}$ and CD4-Cre mice. The Cre gene was detected by PCR using primer 1 (5′-GCATTTCTGGGGATTGCTTA) and 2 (5′-CCCGGCAAAACAG-GTAGTTA); detection of the IL-10 and $gp130^{fl}$ alleles was performed as previously described [5, 27].

Parasite, parasite antigen, egg preparation and animal infection

T. muris initially provided by Dr. K. J. Else (University of Manchester, UK) was maintained in susceptible RAG2$\gamma_c^{-/-}$ mice. Between days 35 and 42 p.i., adult worms were removed from the caecum and incubated in a 6-well plate containing 10 mL RPMI 1640 medium supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin (all from Gibco) at 37°C and 5% CO_2.

Eggs and parasite antigens were collected by centrifugation after 4 h and overnight incubations. Eggs were washed twice and placed for embryonation in water at room temperature in the dark for 40 days. Supernatant containing *T. muris* E/S antigen was concentrated and dialysed against PBS for 3 days. Subsequently, the concentration was determined by Lowry assay. Eggs were stored in water at 4°C. Overnight and 4 h E/S aliquots were stored at 80°C until use.

For experiments 6 to 8-week-old male mice were orally infected with 160–200 eggs in a volume of 200 μL water using a feeding needle. After various time points, mice were sacrificed, worm burdens in the caecae were assessed and MLN cells were isolated for cell culture.

Isolation and culture of MLN cells

At day 14 or day 21 p.i., MLN were removed and a single-cell suspension was prepared in complete medium (RPMI 1640 containing 1% L-glutamine, 5% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 0.1% β-mercaptoethanol (Gibco)). Cells were washed three times, then plated at 5×10^6 cells/mL and restimulated with a final concentration of 50 μg/mL parasite E/S antigen at 37°C, 5% CO_2 for 48 h for cytokine assays or 24 h for FACS analysis. For cytokine assays, supernatants were harvested and stored at 20°C until use.

T-cell isolation and deletion efficiency

In order to analyse the efficiency of the Cre-mediated deletion, spleens were removed from IL-$10^{-/-}$gp$130^{fl/fl}$ CD4-Cre^+ or IL-$10^{-/-}$gp$130^{fl/fl}$ animals and a single-cell suspension was prepared in PBS containing 0.5% BSA (PBS/BSA). Cells were subjected to erythrocyte lysis (BD Biosciences) and then purified using the $CD4^+$ T-cell isolation kit (Miltenyi). Purity of the $CD4^+$ T-cell fraction and deletion efficiency were analysed by FACS.

Flow cytometry

Splenic cells for gp130 staining were subjected to erythrocyte lysis using ammonium chloride and surface-stained with a biotinylated anti-mouse gp130 antibody, followed by incubation with PE-conjugated streptavidin.

Cultures were stimulated with 10 ng/mL PMA and 1 μ/mL ionomycin for the final 4 h and 5 μg/mL brefeldin A (all from Sigma) for the last 2 h. Cells were then stained for surface markers (CD25 and CD4), fixed and permeabilized either using the Fixation/Permeabilization buffer (ebiosciences) for 1 h on ice or fixed with 2% paraformaldehyde for 30 min on ice, followed by permeabilization for 30 min in PBS/BSA containing 0.5% Saponin. Intracellular staining (IL-17, IFN-γ and Foxp3) was finally performed for 30 min at 4°C.

Data were collected on a FACSCalibur (BD Biosciences) and analysed using FlowJo software v6.3.2 (Tree Star). CD25-FITC, CD4-PECy5, IL-17-PE, IFN-γ-APC, PE-conjugated streptavidin were purchased from BD Pharmingen, Foxp3-PE from ebiosciences, and biotinylated anti-mouse gp130 from R&D Systems.

Cytokine assays

Cytokines and chemokines present in the supernatants after *in vitro* restimulation were quantified on a Luminex 100® LiquiChip® Workstation (Qiagen) with Luminex 100® IS Software v2.3 using a mouse cytokine twenty-plex kit (Biosource). IL-22 was measured using the Quantikine Mouse/Rat IL-22 kit (R&D Systems). All procedures were carried out according to the manufacturer's specifications. Data were analysed using LiquiChip® Analyzer software v1.0.2.

Histology

At different time points after infection, caeca were removed and fixed with 4% formalin for 24–48 h. Tissues were processed and paraffin embedded using standard histological techniques. Sections were stained with H&E and were blindly scored 0–3 for severity of inflammation, area involved, ulceration and presence of oedema. For the detection of goblet cells, sections were stained with alcian blue-periodic acid Schiff and the number of goblet cells *per* 100 crypt units was assessed.

Statistical analysis

Statistical analysis was carried out using Student's *t*-test and the Kruskal–Wallis test with Dunns post test for Fig. 5B. Data were always representative of at least two independent experiments. Graphs always show the mean value and the standard deviation. Values of $p < 0.05$ were considered significant.

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Abbreviations: **E/S:** excretory secretory · **p.i.:** post infection · **RA:** rheumatoid arthritis

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