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RAPID COMMUNICATION

Serum interleukin-1 receptor antagonist is an early indicator of colitis onset in Gαi2-deficient mice

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Abstract

AIM: To study the serum concentration of IL-1β, IL-1 receptor antagonist (IL-1Ra) and IL-18 in Gαi2-deficient mice at the age of 6 (healthy), 12 (pre-colitic) and 24 wk (colitic) and in healthy control mice.

METHODS: At the time of killing, serum samples were collected and IL-1β, IL-1Ra and IL-18 levels were measured using enzyme-linked immunosorbent assays.

RESULTS: Serum concentration of IL-1Ra was significantly increased in pre-colitic (median: 524 ng/L; $P = 0.02$) and colitic (450 ng/L; $P = 0.01$), but not in healthy (196 ng/L) Gαi2-deficient mice as compared with controls (217 ng/L). Serum concentrations of IL-1β did not differ between Gαi2-deficient mice and their controls, irrespective of age, IL-18 was significantly increased in colitic, but not in pre-colitic mice compared with controls (510 ng/L *vs* 190 ng/L; $P = 0.05$).

CONCLUSION: The increased serum concentrations of IL-18 and IL-1Ra in established diseases are suggested as markers of ongoing colitis. Interestingly, the significantly increased serum concentration of IL-1Ra in pre-colitic mice is found to be an early marker of disease progression.

Key words: IL-1Ra; IL-18; IBD; Colitis; Mice

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INTRODUCTION

Inflammatory bowel disease (IBD), or ulcerative colitis (UC) and Crohn's disease (CD), is characterized by inflammation in the intestinal tract without any evident infection. The pathogenetic mechanisms underlying the disease induction and progression is still unclear, although clinical studies and animal models have offered insights into the pathogenesis of IBD[1]. It is also of prognostic value in finding analyses enabling early detection of relapses, thus being able to define medical treatment and possibly cease the disease progression at an early stage. Alternatively, if the patients are at a higher risk of relapse, a more intense clinical contact is necessary[2]. Exploring the pathogenesis of IBD and finding early markers of the disease activity are two approaches that might benefit each other. In this context, on the experimental basis, it is of great value to continuously follow animals that are born healthy, therefore displaying a disease-free interval, but pre-disposed for IBD. G protein αi2 deficient mice develop a chronic intestinal inflammation much resembling UC in human beings, including the development of adenocarcinomas[3]. Since the mice are born healthy and develop disease at the age of 12-25 wk, it is possible to study the pathogenesis as well as the effect of interventions initiated before the induction of the disease or during ongoing inflammation. Furthermore, the murine model of Gαi2-deficient mice enables studies on early markers of IBD induction/progression.

The importance of the commensal flora for the induction of experimental IBD is reported in several different models where animals under specific pathogen-free conditions develop intestinal inflammation, while they remain healthy or develop only mild inflammation in a germ-free environment[4-7]. This is true also for Gαi2-deficient mice (Birnbaumer, personal communication). As far as we know, the toll-like receptors (TLRs) form one of the primary sensors of the immune system in detecting bacterial structures. Interestingly, several components of the TLR intracellular (IC) signaling pathway are shared also by interleukin-1 receptor type I (IL-1RI) and the

interleukin-18-receptor (IL-18R). Since these IC signaling mediators, e.g. IL-1 receptor-associated kinase-1 (IRAK-1)[8] and -M (IRAK-M)[9], are highly involved in the regulation of TLR signaling, one might expect an early and important role of IL-1RI and IL-18R in regulating inflammation induced via TLR. IL-18 is a co-stimulator for IFN-γ production[10] and the Gαi2-deficient mice is known to display an immunological dysregulation characterized by high IFN-γ production in intestinal compartments[11]. The main sources of IL-18 in the intestinal tract are intestinal epithelial cells (IEC), macrophages and dendritic cells (DCs)[12-14] The importance of IL-18 for the induction of colitis has previously been shown in the model of 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced colitis in which IL-18-deficient mice do not develop disease, whereas a Crohn's-like disease develops in the wild-type mice[15]. The interleukin-1-receptor antagonist (IL-1Ra) binds to the IL-1RI and blocks the pro-inflammatory function of IL-1 in colitis[16] and neutralization of IL-1Ra results in the exacerbation of colitis[17] Though the IL-1Ra production is significantly increased by IEC during inflammation in CD and UC patients[18], an increased IL-1/IL-1Ra ratio in the intestinal mucosa is still seen in these patients[19]. The spontaneous production of IL-1β is highly increased (30-fold) in colons of Gαi2-deficient mice with colitis as compared with wild-type mice, while the increase in TNF production is more modest (2-fold)[11]. The aim of this study was to analyze the serum concentrations of IL-1β, IL-1Ra, and IL-18 in pre-colitic and colitic Gαi2-deficient mice in order to find early serum markers of colitis.

MATERIALS AND METHODS

Mice

Mice were kept and bred at the animal facility of the Department of Experimental Biomedicine, Göteborg University, Sweden. They were kept under standard conditions of temperature and light, and fed with standard laboratory chow and water *ad libitum*. The procedure of Gαi2 gene disruption has been described in detail elsewhere[3]. Gαi2-deficient mice on a mixed C57BL/6X129SvEv background were backcrossed four generations into 129SvEv and then intercrossed. Homozygous $G\alpha i2^{-/-}$ males were bred with heterozygous females and the offspring were genotyped by polymerase chain reaction (PCR) analysis. Gαi2-deficient mice ($G\alpha i2^{-/-}$) on this background developed colitis between 12 and 25 wk of age, irrespective of sex, while the $G\alpha i2^{+/-}$ similar to $G\alpha i2^{+/+}$ mice stayed healthy. The mice were killed at the age of 6 (healthy), 12 (pre-colitic), and 24 (colitic) wk. At the time of killing, a gross examination of the intestines was performed to look for the presence of colitis. Colitis was scored: 0 = normal; 1 = mild colitis; 2 = moderate colitis and 3 = severe colitis. Blood samples were taken and spleens were weighed. Both male and female mice were included in the study. Heterozygous mice were used as controls throughout the study. The experiments were approved by the Göteborg Animal Ethics Committee.

Cytokine analyses

Serum concentrations of IL-1β and IL-1Ra were estimated using Quantikine mouse immunoassays (R&D Systems, Minneapolis, MN, USA) and IL-18 was measured using a commercially available enzyme immunosorbent assay (MBL, Nagoya, Japan). Detection limits were: IL-1β: 3 ng/L, IL-1Ra: 7 ng/L and IL-18: 25 ng/L. All the assays were performed in accordance with the manufacturer's instructions.

Statistical analysis

Data were analyzed using the non-parametric Mann-Whitney *U* test. A value of $P \leqslant 0.05$ was considered as statistically significant.

RESULTS

Serum IL-18 but not IL-1β is a marker of ongoing colitis

We examined the serum concentrations of IL-18 and IL-1β in the colitic animals as well as their littermate controls. As expected heterozygous controls displayed no or very low serum IL-1β concentration, but also the 24-wk-old Gαi2-deficient mice had serum levels of IL-1β as compared to $G\alpha i2^{+/-}$ controls (median: 18 ng/L *vs* 9 ng/L; ns, not shown). The levels of circulating IL-18 in heterozygous controls were clearly above the detection limit of the assay. However, in contrast to the IL-1β serum levels, the IL-18 concentration was significantly increased in the colitic mice as compared with control animals (Figure 1A). One of the $G\alpha i2^{+/-}$ mice displayed a highly increased IL-18 level as compared with the controls. This mouse appeared healthy and was included in the statistical analysis.

Serum IL-1-receptor antagonist is an early indicator of colitis onset

The serum concentrations of IL-1Ra were analyzed in Gαi2-deficient mice and their littermate controls. In parallel with IL-18, serum IL-1Ra was also easily detected in control animals. In contrast to IL-1β that was not increased in colitic animals, the IL-1Ra concentrations were significantly higher than the controls (Figure 1B). Interestingly, similar to the colitic animals, pre-colitic $G\alpha i2^{-/-}$ mice also had significantly higher serum IL-1Ra levels than their controls. In contrast, serum concentrations of IL-18 and IL-1β were not raised in the 12-wk-old pre-colitic animals as compared with their controls (median 325 ng/L *vs* 190 ng/L; and 10 ng/L *vs* 9 ng/L).

In the 12-wk-old group of pre-colitic $G\alpha i2^{-/-}$ mice, 6 mice did not show any signs of colitis at gross examination, two had mild disease, one moderate and one was judged to have severe colitis. In the 24-wk-old group only one mouse had a normal colon by gross examination, two had mild disease, three moderate and three had severe disease.

The IL-18 concentrations were found higher in colitic than in pre-colitic mice, which was in parallel with a disease progression between 12 and 24 wk of age. However, this was not seen with regard to the serum IL-1Ra concentration. In our previous study Gαi2-deficient cells showed exaggerated cytokine production upon

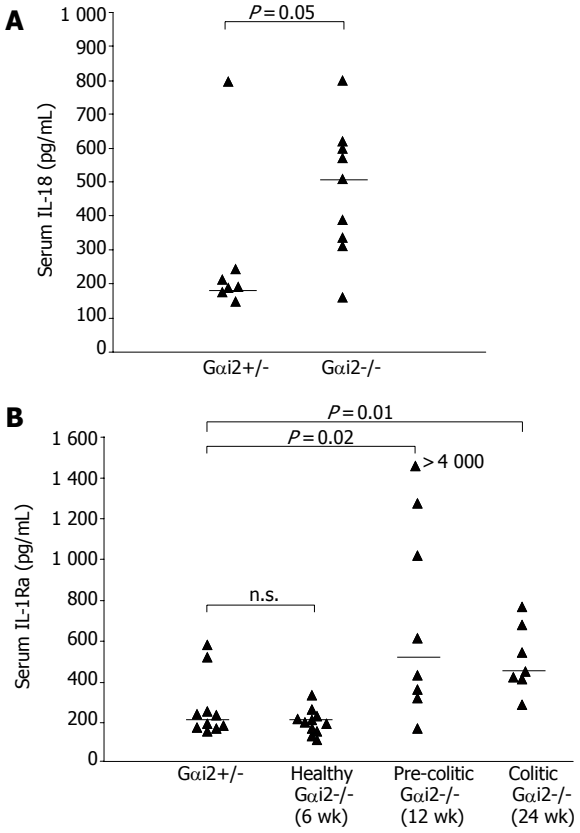

Figure 1 A: Serum IL-18 concentrations in 24-wk-old Gαi2-deficient mice ($n=9$) and their heterozygous littermates (controls; $n=7$). Data were analyzed using the non-parametric Mann-Whitney *U* test. A value of $P \leqslant 0.05$ was regarded as statistically significant. The horizontal bars mark the median values within each group; **B:** Serum IL-1Ra concentrations in 6-wk-old (healthy; $n=11$), 12-wk-old (pre-colitic; $n=8$), and 24-wk-old (colitic; $n=7$) Gαi2-deficient mice and their heterozygous controls ($n=10$). Data were analyzed using the non-parametric Mann-Whitney *U* test. A value of $P \leqslant 0.05$ was regarded as statistically significant. The horizontal bars mark the median values within each group.

stimulation, even if they were derived from a non-colitic genetic background[20]. To examine whether the IL-1Ra concentration was increased upon colitis onset, we also analyzed the serum IL-1Ra concentration in 6-week-old (healthy) Gαi2-deficient mice. The 6-wk-old $G\alpha i2^{-/-}$ mice had similar serum level of IL-1Ra to the $G\alpha i2^{+/-}$ control animals. Thus, serum IL-1Ra concentrations increased upon colitis onset which is not merely a consequence of the Gαi2 deficiency (Figure 1B).

Spleen weight seemed to be also correlated with the stage of the disease. The median weight of 132 mg in 12-wk-old mice, was significantly higher than in $G\alpha i2^{+/-}$ mice (84 mg; $P=0.03$). The 24-wk-old mice displayed a higher spleen weight (222 mg) than not only the controls, but also the 12-wk-old mice ($P=0.03$). Interestingly, the two 12-wk-old mice with severe and moderate colitis judged by gross examination demonstrated the highest spleen weight and the highest serum IL-1Ra concentrations: 271 mg and > 4000 ng/L in mouse with severe colitis and 180 mg and 1276 ng/L in mouse with moderate colitis.

DISCUSSION

We analyzed serum concentrations of IL-18, IL-1β and the IL-1 receptor antagonist in Gαi2-deficient mice on a colitis-prone genetic background. Since mice are born healthy and develop colitis at the age of approximately 12-25 wk, we were able to examine the mice at a "pre-colitic (12 wk of age) and a "colitic" age (24 wk). Our results showed that the serum IL-18 concentration was significantly increased in Gαi2-deficient mice at 24 wk of age, a time when most of the mice displayed moderate or severe colitis, as compared with the controls. At this time-point serum IL-1Ra concentrations were also significantly increased, whereas the serum IL-1β levels were similar in Gαi2-deficient and control mice. The 12-wk-old, "pre-colitic" Gαi2-deficient mice did not display increased serum levels of neither IL-18 nor IL-1β. In contrast, the serum concentration of IL-1Ra was significantly increased in these mice. Our results suggest a potential clinical importance of measuring serum concentrations of IL-18 and IL-1Ra in IBD patients to determine the status of the inflammatory disease. The value of measuring serum IL-18 might be related to confirming ongoing colitis, additional information could be obtained by examining the serum IL-1Ra concentration. In fact, serum concentration of IL-1Ra is an early indicator for the onset of colitis.

We know from previous studies on Gαi2-deficient mice that the production of IL-1β is highly increased locally in the large intestine during ongoing colitis[11]. However, the present study clearly shows that such a dysregulation of the IL-1β production is not reflected in the systemic circulation. In contrast to IL-1β, IL-18 is easily detected in the blood in healthy individuals, of both mouse and man, which enables evaluation of fluctuations in IL-18 concentrations during the disease. The finding of significantly increased IL-18 levels in the 24-wk-old colitic mice is not very surprising, inasmuch as the intestinal inflammation of the Gαi2-deficient mice is characterized by high IFN-γ production, with IL-18 being a co-stimulator for IFN-γ production. It has been previously shown that serum IL-18 was higher in patients with CD than healthy blood donors[21]. A recent report also showed increased plasma IL-18 level in patients with moderate or severe UC, but not in patients with mild disease[22]. Our results confirm a role of IL-18 levels measurement in established colitis. However, it seems that IL-18 is not as sensitive as IL-1Ra in indicating onset of colitis, or perhaps not as useful as an early indicator of a relapse as compared with IL-1Ra. We found that although there were clearly detectable levels of IL-1Ra in young healthy Gαi2-deficient mice, the levels were similar to what was found in the control mice. In contrast, the 12-wk-old "pre-colitic" Gαi2-deficient mice displayed significantly higher serum IL-1Ra concentrations than their controls. In the healthy mucosa, IEC is capable of producing IL-1Ra[18] which might be one of the sources for the serum IL-1Ra detected in healthy $G\alpha i2^{-/-}$ mice and their $G\alpha i2^{+/-}$ littermates. During intestinal inflammation, IEC increased the production of IL-1Ra, but a decreased IL-1Ra:IL-1 ratio was nevertheless seen[23]. One reason for finding IL-1Ra but not IL-1β in the circulation at the onset and in established colitis might simply be that the receptor antagonist is produced excessively as compared with the cytokine and is therefore more easily detected. Another explanation could of course be that the IL-1Ra is systemically derived. Although the visceral

organs of Gαi2-deficient mice were previously screened microscopically without finding any abnormalities, except the colon[3], splenomegaly seems to be correlated with disease progression in Gαi2-deficient mice. One possible tissue origin of serum IL-Ra in healthy individuals, except IEC, is the adipose tissue[24,25]. An increased serum IL-1Ra concentration in the older Gαi2-deficient can hardly be related to increased BMI. In contrast from previous results we found that serum leptin, also originating from white adipose tissue, is decreased upon colitis onset in Gαi2-deficient mice[26]. Furthermore, the 12-wk-old mouse displaying severe colitis had a substantial weight reduction as compared with the other mice in the group and still highly increased serum IL-1Ra concentration.

In conclusion, our results show that while circulating IL-18 and IL-1Ra are markers of established intestinal inflammation, IL-1Ra is also an early indicator of onset of colitis in Gαi2-deficient mice. We therefore suggest that IL-1Ra might be a useful serological marker of disease progression in IBD at an early stage as well as at a relapse.

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