Cytokine 69 (2014) 68–74

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Immune-mediated experimental arthritis in IL-33 deficient mice

Dominique Talabot-Ayer [a,b], Praxedis Martin [a,b], Christian Alexander Seemayer [c], Solenne Vigne [a,b], Céline Lamacchia [a,b], Axel Finckh [a], Essia Saiji [d], Cem Gabay [a,b], Gaby Palmer [a,b,*]

[a] Division of Rheumatology, Department of Internal Medicine, University Hospital, Geneva, Switzerland
[b] Department of Pathology and Immunology, University of Geneva School of Medicine, Geneva, Switzerland
[c] Novartis Pharma AG, Basel, Switzerland
[d] Department of Clinical Pathology, University Hospital, Geneva, Switzerland

ARTICLE INFO

Article history:
Received 9 July 2013
Received in revised form 18 March 2014
Accepted 12 May 2014

Keywords:
Arthritis
Cytokine
Immune response
Joint inflammation

ABSTRACT

Previous work suggested implication of the interleukin (IL)-1 family cytokine IL-33, signaling through its receptor ST2, in the pathogenesis of human and mouse arthritis. In this study, we directly investigated the role of endogenous IL-33 in antigen-induced arthritis (AIA) and collagen-induced arthritis (CIA) using IL-33 KO mice.

AIA was induced by injection of methylated bovine serum albumin (mBSA) into knee joints of previously immunized mice. CIA was induced by immunization with bovine type II collagen. Disease severity was evaluated by clinical and histological scoring and cellular immune responses were assessed in cultured draining lymph node cells. Our results indicate that the development of AIA or CIA, as assessed by clinical or histological evaluation, is not impaired in IL-33 deficient mice. We did not observe any consistent modifications in humoral or cellular immune responses in IL-33 KO mice, although IL-33 deficiency enhanced antigen-specific IFN-γ production, proliferation or IgG2a titers in some experiments, suggesting that endogenous IL-33 may contribute to shaping the adaptive immune response. In conclusion, our data suggest that IL-33 plays a modifying rather than a pivotal role in disease development in two models of immune-mediated arthritis.

1. Introduction

Interleukin (IL)-33 is the most recently discovered member of the IL-1 cytokine family (see ([1]) for review). Like pro-IL-1α, IL-33 is a dual function protein, displaying both nuclear and extracellular effects [2–5]. The latter are mediated by its binding to an IL-1 receptor family member called ST2 [5], which is expressed on many cells of the innate and adaptive immune system. The hypothesis that emerged over the last years is that IL-33 is primarily expressed as a nuclear protein, which is released upon cell damage or stress to act as an alarmin [6,7]. In agreement with this notion, recent studies have emphasized a role for IL-33 in shaping both innate and adaptive immune responses, thereby contributing to host defense and immunopathology [8–11].

Several studies suggested involvement of the IL-33/ST2 axis in the inflammatory process in arthritis. Indeed, IL-33 and ST2 are expressed in human rheumatoid arthritis (RA) synovium [3,12–14] and expression of IL-33 is induced by pro-inflammatory stimuli in cultured human synovial fibroblasts [12,13,15,16]. IL-33 levels are increased both in serum and synovial fluid of RA patients as compared to healthy donors or osteoarthritis patients, and decrease with successful treatment, although correlation with disease activity was not observed in all studies [14,15,17–20].

In mice, different experiments using the soluble(s) ST2 decoy receptor, blocking anti-ST2 antibodies, injection of recombinant IL-33 or ST2 knockout (KO) mice, have provided indirect evidence for a proinflammatory effect of IL-33, signaling via ST2, in experimental models of arthritis that are dependent on antigen-specific immune responses, such as collagen-induced arthritis (CIA) and antigen-induced arthritis (AIA) [12,13,21,22]. IL-33 KO mice have recently become available, allowing for direct study of the role of IL-33 *in vivo* [9,10,23]. However, first data published using IL-33 KO mice led to divergent conclusions concerning the importance

Abbreviations: AIA, antigen-induced-arthritis; CII, type II collagen; CFA, complete Freund's adjuvant; CIA, collagen-induced arthritis; DLN, draining lymph node; IFN, interferon; IL, interleukin; KO, knockout; mBSA, methylated bovine serum albumin; RA, rheumatoid arthritis; sST2, soluble ST2; WT, wild-type.

* Corresponding author at: Department of Pathology and Immunology, Centre Médical Universitaire, 1 rue Michel-Servet, 1211 Geneva 4, Switzerland. Tel.: +41 22 379 57 68; fax: +41 22 379 57 46.

E-mail address: Gaby.Palmer@unige.ch (G. Palmer).

http://dx.doi.org/10.1016/j.cyto.2014.05.007
1043-4666/

Table 1
Sequences of primers used for RT-qPCR quantification of joint cytokine and chemokine expression.

IL-6 forward	5′-TGAACAACGATGATGCACTTGCAGA-3′
IL-6 reverse	5′-TCTGTATCTCTCTGAAGGACTCTGGCT-3′
TNF-α forward	5′-AGTTCTATGGCCCAGACCCT-3′
TNF-α reverse	5′-GTCTTTGAGATCCATGCCGT-3′
IL-1β forward	5′-TGTGAAATGCCACCTTTTGA-3′
IL-1 β reverse	5′-GTGCTCATGTCCTCATCCTG-3′
Cxcl-1 forward	5′-ACTCAAGAATGGTCGCGAGG-3′
Cxcl-1 reverse	5′-GTGCCATCAGAGCAGTCTGT-3′
18S forward	5′-GTAACCCGTTGAACCCCATT-3′
18S reverse	5′-CCATCCAATCGGTAGTAGCG-3′

of endogenous IL-33 in adaptive immunity [9–11,24], so that this issue warrants further investigation. Furthermore we recently obtained results indicating that joint inflammation in the passive K/BxN serum transfer-induced model of arthritis is not modified by IL-33 deficiency [25].

In the present study, we examined the role of endogenous IL-33 in AIA and CIA using IL-33 KO mice backcrossed respectively to the C57BL/6 and the DBA/1 backgrounds. Our observations suggest that IL-33 deficiency has no major effect on the development of arthritis in these models.

2. Materials and methods

2.1. Mice

C57BL/6 WT mice were obtained from Elevages Janvier (Le Genest-St-Isle, France). IL-33 KO were generated at Amgen Inc. and backcrossed to the C57BL/6 background for 6 generations by speed congenics [25]. They were considered to be 100% congenic based on analysis of a 377 SNIP panel (Taconic, Hudson, NY). For AIA experiments, local colonies of WT and IL-33 KO mice were established at the Centre Médical Universitaire in Geneva. For CIA, IL-33 KO mice and their control WT littermates were obtained by interbreeding of heterozygous IL-33 KO mice backcrossed for 7 generations from a mixed 129Sv × C57BL/6 to a DBA/1 background. Mice were housed under conventional conditions, and water and standard laboratory chow were provided ad libitum. Institutional approval was obtained for all animal experiments (Geneva Cantonal Authority for Animal Experiments, licenses 31.1.1005/3402/2, 3402/2-C, 3402/2-R, 3540/2 and 3880/1).

2.2. Antigen-induced arthritis (AIA) and immune response to mBSA

Adult male C57BL/6 WT and IL-33 KO mice were immunized intradermally at the base of the tail with 100 μg of methylated bovine serum albumin (mBSA; Fluka, Buchs, Switzerland), emulsified in complete Freund's adjuvant (CFA, Difco, Basel, Switzerland) containing 5 mg/ml Mycobacterium tuberculosis. On day 7, a booster injection of 100 μg mBSA in incomplete Freund's adjuvant (Difco) was given at the base of the tail. In one experiment, mice were sacrificed on day 21 after the first immunization to assess the immune response to mBSA in the absence of arthritis. In the other experiments, arthritis was induced on day 21 by intra-articular injection of 100 μg mBSA in 10 μl PBS into the left knee joint of mBSA-immunized mice, the right knee being injected with sterile PBS alone. Mice were sacrificed 4 or 8 days after induction of arthritis. The development of arthritis was followed by measuring ^{99m}Technetium (Tc) uptake in the knees on days 1, 3 and 7 after intra-articular mBSA injection, as previously described [26].

2.3. Collagen-induced arthritis (CIA)

CIA was induced in adult male DBA/1 IL-33 KO and their WT littermates by immunization with bovine type II collagen (CII) in

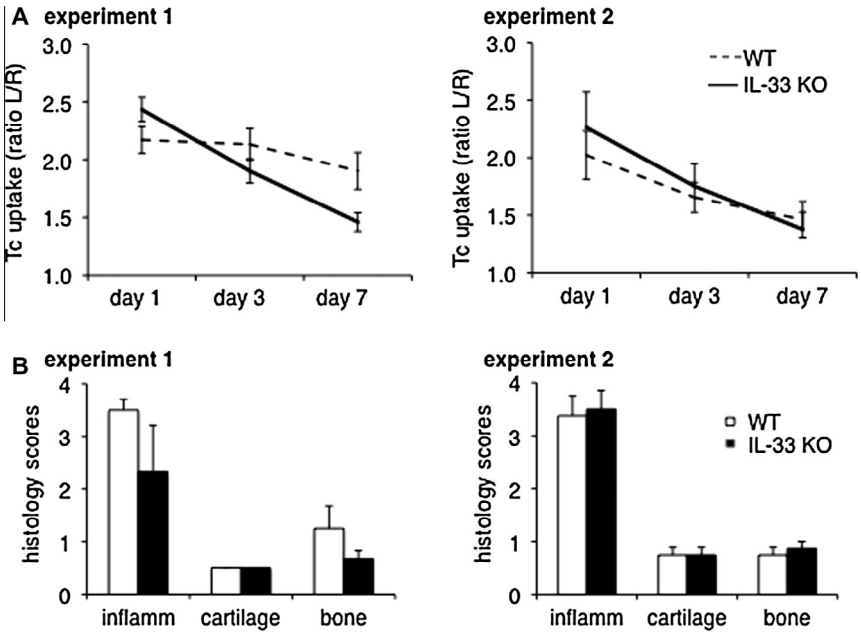

Fig. 1. Severity of AIA in IL-33 KO and WT mice. (A) Joint inflammation on days 1, 3 and 7 after intra-articular mBSA injection in IL-33 KO and WT mice in the C57BL/6 background. Results are expressed as the ratio of ^{99m}Tc uptake in the arthritic over the non-inflamed knee. Data obtained in two independent experiments are shown as the mean + SEM for each group of mice. Experiment 1 (left panel): IL-33 KO mice (n = 9 for days 1 and 3, n = 4 for day 7; black line) and WT C57BL/6 mice (n = 8 for days 1 and 3, n = 4 for day 7; dashed line). Experiment 2 (right panel): IL-33 KO mice (n = 4; black line) and WT C57BL/6 mice (n = 4; dashed line). No significant differences were observed between the groups. (B) Histological scores for arthritis severity, as assessed by evaluating inflammation (inflamm), cartilage erosion and bone erosion on day 8 after intra-articular mBSA injection, are shown as the mean + SEM. Experiment 1 (left panel): IL-33 KO mice (n = 3; black columns) and WT C57BL/6 mice (n = 4, open columns). Experiment 2 (right panel): IL-33 KO mice (n = 4; black columns) and WT C57BL/6 mice (n = 4, open columns). No significant differences in histological scores were observed between the groups.