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Generation of and characterization of anti-IL-11 antibodies using newly established *Il11*-deficient mice

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ABSTRACT

Interleukin (IL)-11 belongs to the members of the IL-6 family of cytokines and is involved in a variety of biological responses, including hematopoiesis, bone development, and carcinogenesis. However, the cellular sources of IL-11 and regulation of IL-11 expression under physiological and pathological conditions are not fully understood. One of the causes to prevent characterization of IL-11 in vivo is due to the lack of reliable antibodies that detect IL-11 by immunohistochemistry. Moreover, although mice lacking *Il11ra* have been generated and extensively characterized, *Il11*-deficient mice have not been characterized yet. Here we generated two anti-IL-11 antibodies that blocked biological activities of IL-11 and detected IL-11 by immunohistochemistry, respectively. One clone of anti-IL-11 antibodies blocked IL-11-, but not IL-6-induced cell proliferation and IL-11-induced phosphorylation of STAT3 of an IL-11-dependent cell line. Moreover, we used recently established *Il11*-deficient mice to test the specificity of anti-IL-11 antibodies for immunohistochemistry. Another clone of anti-IL-11 antibodies stained stromal cells surrounding tumors of the colon of wild-type, but not *Il11*-deficient mice following treatment with Azoxymethane plus dextran sulfate sodium. Together, these newly developed anti-IL-11 antibodies provide a better understanding of the functions of IL-11 in vivo under various physiological and pathological conditions.

1. Introduction

Interleukin (IL)-11 belongs to the members of the IL-6 family of cytokines and the IL-11 receptor is composed of IL-11Rα and gp130 [1,2]. While IL-11Rα mediates binding to IL-11, gp130 transmits the signals. The binding of IL-11 to the IL-11 receptor induces the activation of JAK that subsequently phosphorylates STAT3. Then, phosphorylated STAT3 goes into the nucleus and activates the transcription of various target genes [1,2].

IL-11 regulates many biological responses such as hematopoiesis, bone development, and tissue repair [1]. IL-11 has been shown to be involved in the development of colitis-associated colorectal cancer in human and various tumor models of the colon in mice [3–5]. We previously reported that IL-11 is produced in a ROS-

Abbreviations: CRISPR, Clustered regularly interspaced short palindromic repeats; STAT, Signal transducer and activator of transcription; AOM, Azoxymethane; DSS, dextran sulfate sodium; IL, Interleukin; JAK, Janus kinase.

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dependent manner and promotes cell proliferation of hepatocytes [6]. Moreover, we also reported that one of the electrophiles, 1,2-Naphthoquinone (1,2-NQ), induces IL-11 production, and IL-11 attenuates 1,2-NQ-induced intestinal damage [7]. Although accumulating studies have indicated that IL-11 is produced by stromal cells, hematopoietic cells, or epithelial cells in a context-dependent manner [5,6,8–11], it is not unclear which types of cells predominantly produce IL-11 under physiological and pathological condition. To address this issue, here we generated monoclonal antibodies against murine IL-11 (mIL-11) that had neutralizing activity of mIL-11 and detected mIL-11 by immunohistochemistry (IHC). These antibodies might provide a better understanding of the functions of IL-11 in various physiological and pathological conditions in vivo.

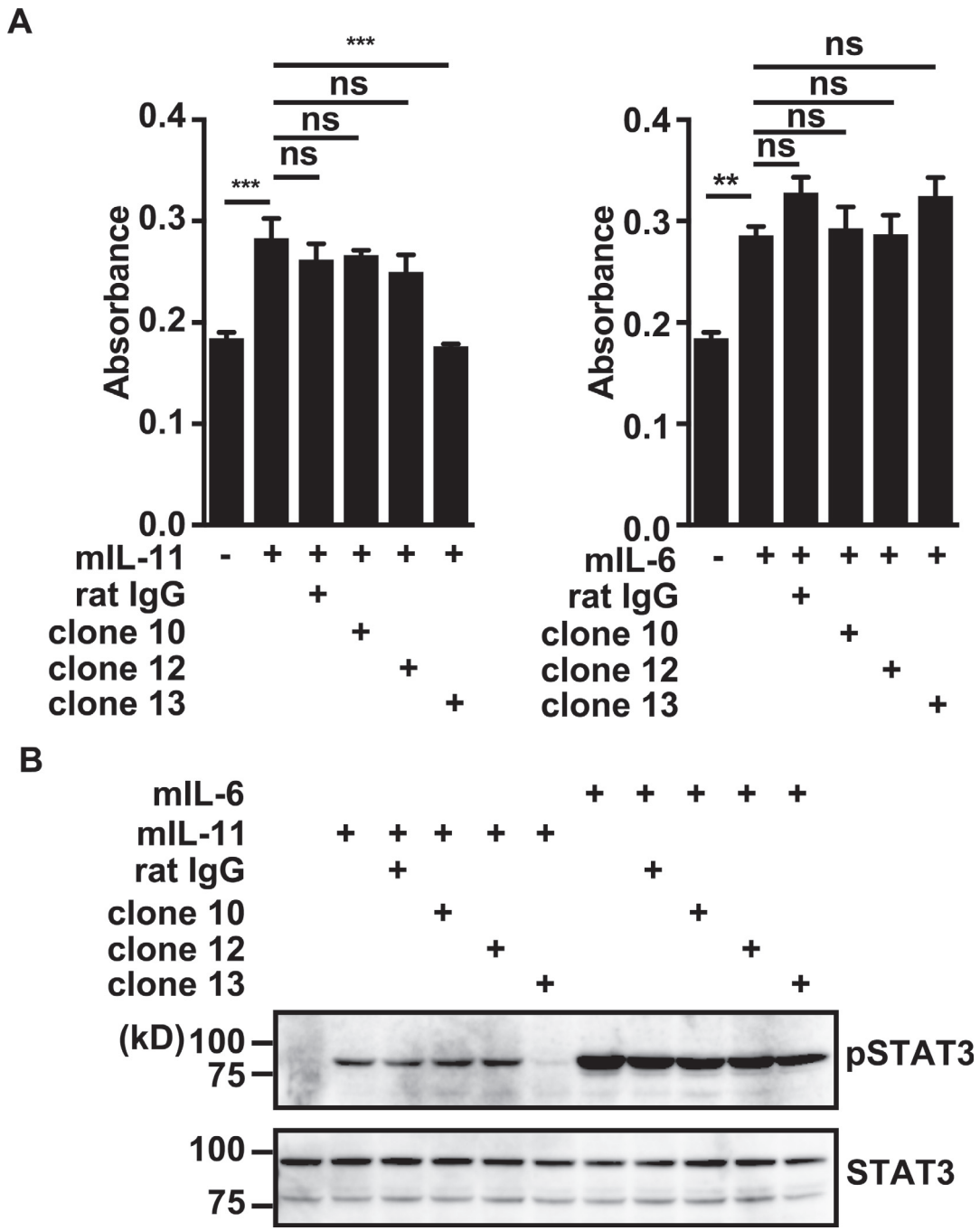

Fig. 1. Characterization of anti-mIL-11 antibodies. (**A**) T1165 cells were stimulated with mIL-11 (left) or mIL-6 (right) in the absence or presence of control rat IgG, or the indicated clones of anti-mIL-11 antibodies. Proliferation of cells was determined by WST assay. Statistical significance was determined by the one-way ANOVA test. Results are means ± SD of triplicate samples and representative of two independent experiments. $^{**}P < 0.01$; $^{***}P < 0.001$, ns; not significance. (**B**) mIL-11 or mIL-6 were preincubated with the absence or presence of control rat IgG, or the indicated clones of anti-mIL-11 antibodies for 30 min. Then, CT-26 cells were stimulated with pretreated mIL-11 or mIL-6 for 10 min. Phosphorylation of STAT3 was determined by immunoblotting with anti-pSTAT3 antibody using total cell lysates.

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