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Sumiko Iho

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LETTERS TO THE EDITOR

Type I IFN Synthesis in Plasmacytoid Dendritic Cells

Kerkmann et al. (1) recently reported two distinct pathways for IFN-α induction in human plasmacytoid dendritic cells (PDC), one dependent on and one independent of the IFNR-mediated feedback loop. However, the mechanisms by which the IFNR-mediated pathway is initiated were not mentioned. Concerning this matter, and because they did not make reference to our study (2), we provide here our results: Human PDC constitutively express IFN regulatory factor-7, and its enhancement and the nuclear-translocation by CpG DNA is preceded by p38 mitogen-activated protein kinase-dependent phosphorylation of STAT1. This pathway is triggered in a manner independent of autocrine response to IFN-$\alpha\beta$, thereby leading to the IFNR-mediated response to produce a vast amount of IFN-α. The sequence of the active motif we used is GACGATCGTC, which also exists in CpG-A (ODN 2216) that Kerkmann et al. used. This motif was originally reported by Kuramoto et al. (3) in mice, as possessing a potent immunostimulatory activity especially when flanked by poly (G) sequence; and has now been revealed to have outstanding activity in humans, as well. The ability of this sequence to directly activate the IFNR-independent pathway would help in elucidating the mechanisms of IFN-α production in PDC and designing the CpG DNA as a therapeutic drug.

Sumiko Iho

Department of Immunology and Medical Zoology
Fukui Medical University
Fukui, Japan

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Cannabinoid-Induced Activation of ERK and AKT in Mast Cells May Be Mediated by Intracellular NO Production

Samson and colleagues elegantly demonstrated the coexpression of CB1/CB2 receptors in a mast cell line (RBL2H3) and noted that while CB1 was functional in the suppression of serotonin release, CB2 was the predominant mediator of cannabinoid-induced AKT and ERK kinase phosphorylation (1). We recently reported that L-NAME, an inhibitor of NO synthase (NOS), prevented the inhibitory effect of exogenous (CP 55,940) and endogenous (2-arachidonylglycerol) cannabinoids on the Ag-induced activation of guinea-pig mast cells (2). The effect of cannabinoids was reasonably CB2 mediated, since it was completely reverted by a selective CB2 receptor antagonist (SR144528) (2, 3). CP 55,940 and 2-arachidonylglycerol increased the production of nitrites and cGMP, measured in guinea-pig mast cells supernatants, and promoted the intracellular expression of the inducible isoform of NOS (iNOS), as shown through a Western blot analysis (manuscript in preparation). Since NO involvement in AKT and ERK activation has been shown in several cell types (4, 5), we wonder whether the cannabinoid-induced phosphorylation of AKT and ERK kinases, clearly showed by Samson et al., may occur via a NO/cGMP dependent pathway. In our opinion, a set of experiments performed in the presence or in the absence of inhibitors of NO and cGMP generation may be of interest in the understanding of the multiple transcriptional events induced by cannabinoid exposure.

Alfredo Vannacci, Lucia Giannini, Emanuela Masini, and Pier Francesco Mannaioni

Department of Preclinical and Clinical Pharmacology
University of Florence
Florence, Italy

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IL-4 Is Not a Key Profibrotic Cytokine in Bleomycin-Induced Lung Fibrosis Model

We read with interest the publication of Huaux et al. (1) concerning the dual roles of IL-4 in lung injury and fibrosis. In another recent study (2), bleomycin was injected intratracheally into three groups of C57BL/6JU mice: transgenic animals with overexpression of IL-4, IL-4-deficient mice, and wild-type mice. Lung fibrosis was evaluated at

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14 days by hydroxyproline measurements and by quantitative image analysis of fibrosis fraction and alveolar wall area fraction. In the early phase, similar to Huaux et al. (1), there was no difference between IL-4-deficient and wild-type mice in bronchoalveolar cellularity, hydroxyproline, and fibrosis scores (2). However, since hydroxyproline and fibrosis scores were significantly lower in transgenic animals with overexpression of IL-4 as compared with IL-4-deficient mice, IL-4 does not seem to be a key profibrotic cytokine in the murine model of bleomycin-induced lung fibrosis.

Gabriel Izbicki and Raphael Breuer

Lung Cellular and Molecular Biology Laboratory
Institute of Pulmonology
Hadassah University Hospital and
The Hebrew University-Hadassah Medical School
Jerusalem, Israel

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The Authors Respond

Izbicki et al. (1) limited their study mostly to the early response to bleomycin, focusing on the day 14 time point, while our study (2) extended out to day 28. They also used a higher single dose of bleomycin (0.06 mg/mice or 0.06 U/mice, vs 0.02 and 0.05 in our study) to induce the model in their study. At this high dose (0.06 U/mouse) we lost the majority of our animals at day 21, which may account for the focus on day 14 in their study. They did present limited data at day 21 for the IL-4 transgenic mice (IL-4 TG). However, in contrast to what they described in the text, Table I of their paper (1) suggested significant fibrosis equivalent to that in wild-type mice. Nevertheless, the suggestion that overexpression of IL-4 could be protective against early injury agrees with our data showing that IL-4 deficiency caused higher mortality at the high dose of bleomycin (0.05 U/mouse). Nevertheless it would be difficult to directly compare their results using IL-4 TG mice with our studies, which did not use this strain of mouse.

However, based on the use of similar IL-4 knockout mice, our results appear to agree with respect to the suggestion of a more intense acute response to high dose bleomycin in the absence of IL-4. However if these IL-4-deficient mice were allowed to survive the acute phase by using a lower dose of bleomycin, then some protection from fibrosis became evident. Since Izbicki et al. focused on the day 14 time point with an even higher dose of bleomycin (which would have caused significant mortality by day 21), they would have and did miss the protective effects of IL-4 deficiency at the later stages when fibrosis would be maximal. Hence their conclusion based on their limited findings would be appropriate.

When all the available data are taken together, however, it is clear that the role of IL-4 in this model is more complex than their limited study has allowed them to conclude. The extended findings in our study varying the intensity of injury and analysis at later time points suggest that its role is critically time (or disease stage) dependent. Thus it appears to play anti-inflammatory/immunosuppressive roles acutely at doses likely to be present in wild-type mice, but a pro-fibrotic role at later stages if the animals were allowed to survive the initial acute response. This is not unique to IL-4 since IFN-γ also has similar complex albeit opposite roles. Similarly, TNF-α has effects on fibrosis that are dose dependent, with a "normal" dose being profibrotic, while its overexpression appears to attenuate fibrosis (3). Thus it is probably overly simplistic to state that "IL-4 is not a key profibrotic cytokine" in the bleomycin model.

Sem H. Phan and François Huaux

Department of Pathology
University of Michigan Medical School
Ann Arbor, MI 48109

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