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Constitutive expression of IL-2Rbeta chain and its effects on IL-2-induced vascular leak syndrome

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Abstract

IL-2-induced vascular leak syndrome (VLS) is an important mechanism explaining the toxic effects of this cytokine and limiting its therapeutic use. We previously characterized a mouse model of IL-2-induced pulmonary VLS used to demonstrate that NK lymphocytes are involved in early/acute phase VLS (after one IL-2 injection). We also showed that NK cells and polymorphonuclear neutrophils (PMN) are involved in the late/chronic phase of the syndrome (after four daily IL-2 injections). In this study we use our mouse model to evaluate the role played by the IL-2 receptor (IL-2R) in VLS induction. Mouse and human IL-2R are different since the mouse IL-2Rbeta chain does not recognize IL-2. Here, we compare the acute and late VLS responses in human IL-2Rbeta transgenic and C57BL/6 wild type mice. Parameters linked to early phase VLS (bronchoconstriction and PMN mobilization) are enhanced in human IL-2Rbeta transgenic mice. By contrast, parameters used to measure late events (protein leakage and edema) are similar in human IL-2Rbeta transgenic mice and C57BL/6 wild type animals. However, after four IL-2 injections, the cellular content of the bronchoalveolar lavage fluids was different between the two types of animals. This study also characterizes a humanized animal model that could be further used to study human IL-2 activity and side effects in vivo.

Keywords: Human IL-2Rbeta transgenic mice; IL-2; IL-2 receptors; Vascular leak syndrome (VLS)

1. Introduction

Interleukin-2 (IL-2) exerts a broad spectrum of effects on the immune system and plays a crucial role in regulating both immune activation and homeostasis [1–4]. Clinical trials using human recombinant interleukin-2, alone or in combination with the adoptive transfer of lymphokine-activated killer cells (LAK), has been shown to result in tumor regression in 25–30% of patients with metastatic melanoma or renal carcinoma [5,6]. Intermittent IL-2 administration is also used in HIV-infected patients in combination with highly active antiretroviral therapy (HAART). This association restores sustained, protective levels of $CD4^+$ T lymphocytes [7–11]. However, the therapeutic use of IL-2 is restricted by its dose-dependent toxicity. Its side effects stem mainly from the development of vascular leak syndrome (VLS). IL-2-induced VLS is characterized by increased vascular permeability and decreased microcirculatory perfusion leading to interstitial edema and multiple organ failure [12,13].

In a recent paper we addressed the cellular mechanisms involved in IL-2-induced VLS and described a mouse model of IL-2-induced pulmonary VLS. This was used to analyze both the early events (bronchoconstriction and sequestration of polymorphonuclear neutrophils) and the late events (modifications in the cell and protein contents of bronchoalveolar lavages, followed by edema) that characterize this lung injury [14]. Early/acute events were observed after one IL-2 injection

Abbreviations: AUC, area under the curve; BALF, bronchoalveolar lavage fluid; MPO, myeloperoxidase activity; PMN, polymorphonuclear neutrophils; VLS, vascular leak syndrome.

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whereas late/chronic events were measured after four IL-2 injections. By comparing the results obtained between C57BL/6 and Recombinase-activating gene 2 −/− mice, we showed that B, T and NKT lymphocytes are not required for IL-2-induced pulmonary VLS. By contrast, results obtained with double KO mice (Recombinase-activating gene 2 −/− //IL-15 −/−) demonstrated that NK cells are involved in both the early and late phases of the syndrome. Furthermore, granulocyte depletion was entirely effective in protecting mice from the late events of IL-2-induced pulmonary VLS, thereby demonstrating that NK lymphocytes and PMN are both critical for IL-2-induced pulmonary VLS late events [14].

IL-2 responsiveness is in part controlled by the expression of interleukin-2 receptors (IL-2R). In humans, two types of functional receptor have been described. The high affinity receptor is made of three chains (hIL-2Ralpha, hIL-2Rbeta and hIL-2Rgamma$_c$) [15–21]. Intermediate affinity IL-2R is composed of the association of hIL-2Rbeta and hIL-2Rgamma$_c$ chains, which are selectively expressed by NK cells and monocytes, respectively [22]. Mice lack intermediate-affinity IL-2R and IL-2 responsiveness in these animals is entirely dependent on IL-2Ralpha expression [23,24]. Therefore, the IL-2R system is different in humans and mice and the animal model may not be the most appropriate for studying the in vivo effects of human IL-2.

Here we further investigate the mechanisms involved in IL-2-induced VLS and used previously characterized human IL-2Rbeta transgenic mice for our experiments [25]. In these animals, the H-2k promoter supports constitutive expression of human IL-2Rbeta chain. We have verified that this chain is expressed by all cells of the immune system and that it did not modify the pattern of expression of mouse IL-2R chains [26]. The expression of human IL-2Rbeta chain in the presence of endogenously expressed murine IL-2Rgamma$_c$ chain is sufficient to obtain a functional intermediate-affinity IL-2R [23,27,28]. These animals were used to analyze the role of the IL-2R complex in the early and late events that characterize the toxic pulmonary effects of human IL-2. Based on our results we also suggest that the human IL-2Rbeta transgenic mouse model may be useful for future studies aimed at evaluating the therapeutic index of human IL-2 or human IL-2 mimetics.

2. Materials and methods

2.1. Inducing the early and late phases of vascular leak syndrome

Mice were injected i.v. with 10 μg of IL-2 (180,000 IU Aldesleukin, Chiron B.V., Amsterdam). Control mice were injected i.v. with NaCl (0.9%). Acute responses were evaluated 2 h after the first IL-2 injection. Late responses were measured after four daily i.v. injections and the different biological parameters were analyzed at the times indicated.

Mice were 6–9-week-old males. Wild type C57BL/6 mice were purchased from "CNRS CDTA" (Orléans, France). Human IL-2Rbeta transgenic animals on C57BL/6 background [25], were bred at the Pasteur Institute with standard laboratory feed and water ad libitum.

2.2. Evaluation of broncho-pulmonary hyper-reactivity

Airway resistance was measured by barometric plethysmography using unrestrained conscious mice placed in a plethysmographic chamber (Buxco Electronics, Sharon, USA). Respiratory parameters in each animal were measured in response to the aerosolization of 100 mM metacholine in sterile 0.9% NaCl for 20 s. Responsiveness to methacholine was determined 2 h after the NaCl injection or IL-2 challenge. Resistance was expressed as enhanced pause (Penh) [29]. The means of three results per min for 10 min were calculated. The results were presented as cumulated "areas under the curve" (AUC), giving a quantitative representation of the results obtained over the 10 min test period.

2.3. Myeloperoxidase (MPO) activity of the lung

After bronchoalveolar lavage, lungs were perfused with 5 ml of Isoton injected into the right ventricle then stored at −20 °C pending MPO activity measurements. Briefly, lung tissues were homogenized using a Potter homogenizer (Poter-Elvejhem glass homogenizer, Thomas, Philadelphia, PA) in 1 ml of PBS at room temperature. Homogenates were centrifuged at 10,000 × g for 10 min at 4 °C. Cell pellets were suspended in 1 ml of PBS-HTAB (0.5%); 1 mM EDTA before Potter lysis and centrifugation at 10,000 × g for 10 min at 4 °C. One hundred microliters of the supernatant were diluted with 2 ml of HBSS, 200 μl of PBS–HTAB–EDTA, 100 μl of 1.25 mg/ml odianisidine dihydrochloride (Sigma, France) and 100 μl of 0.05% H_2O_2 (Sigma, France). After 15 min at 37 °C the reactions were stopped with 100 μl of 1% sodium azide (Sigma, France). Absorbance was determined on a spectrophotometer at 460 nm.

2.4. Analysis of BALF cell and protein content

Alterations into the cell and protein contents of bronchoalveolar lavage fluids (BALF) were studied by conventional procedures. Briefly, mice were deeply anaesthetized with 0.5 ml of urethane (1.5 g/kg, Sigma, France) injected intraperitoneally. The trachea was cannulated and the airways rinsed five times with 0.5 ml of PBS. The fluid was withdrawn and stored on ice.

Total cell counts were evaluated in 400 μl samples diluted in 20 ml of Isoton (Beckman Diagnostics, Krefeld, Germany) plus Zap-Oglobin (Beckman, Villepinte, France) to lyse the cells. Total nuclei were counted using an electronic coulter counter (Coulter Electronics, Luton, UK). Cytospin preparations of the BALF were stained with Diff-Quick (Dade Behring, Marburg, Germany). A differential count of 200 cells was performed using standard morphological criteria.

The remaining BALF was centrifuged and the supernatant collected and stored at −20 °C for total protein measurements. Bradford stain was added to 50 μl of the supernatant diluted in 950 μl of H_2O plus 250 μl of Bradford solution (Biorad SA,