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The quinoline-3-carboxamide paquinimod (ABR-215757) reduces leukocyte recruitment during sterile inflammation: Leukocyte- and context-specific effects

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ABSTRACT

Quinoline-3-carboxamides (Q-compounds) are currently in clinical development for both autoimmune disease and cancer. We have previously shown that the Q-compound paquinimod (ABR-215757) significantly ameliorates disease symptoms in several mouse models of human inflammatory disease. Considering that recruitment of inflammatory cells into tissue is a common denominator of these models, we have in this report investigated whether paquinimod would interfere with cell accumulation during sterile peritoneal inflammation. To mimic the cell recruitment elicited by tissue injury, we used necrotic cells to induce the acute inflammatory response. We show that per oral treatment with paquinimod significantly reduced the accumulation of Ly6C^{hi} inflammatory monocytes and eosinophils, but not neutrophils, in this model, and that this correlated with reduced number of such cells also in the omentum. Treatment also reduced the accumulation of these cell populations at a subcutaneous site of inflammation. In alum-induced inflammation, however, neutrophils were the dominant cell population and paquinimod failed to reduce the accumulation of inflammatory cells. Taken together, our results indicate that paquinimod selectively inhibits cell recruitment during acute sterile inflammation, but that this effect is context-dependent. These data have important implications for the understanding of the mechanism of action of Q-compounds in both pre-clinical and clinical settings.

1. Introduction

Both tissue injury and infection can induce an acute inflammatory response. The application of irritants or bacterial products in the peritoneal cavity has long been used as a standard method for collecting macrophages from mice. The induction of experimental peritonitis in such settings has been widely used as a model to study the accumulation of inflammatory cells during sterile conditions.

The steady state peritoneal cavity contains a self-renewing resident macrophage population [1,2]. These cells are activated upon injury and infection and contribute significantly to the recruitment of inflammatory leukocytes during peritoneal inflammation [3,4]. Mesothelial cells express toll-like receptors (TLRs) and are also engaged in the response by producing various inflammatory cytokines and chemokines (reviewed in [5]). The inflammatory cells entering the peritoneal cavity originate from blood vessels in milky spots in the greater omentum, mesenteric blood vessels and blood vessels at other mesothelial sites [6,7]. In the initial phase, neutrophils and CX_3CR1^+ patrolling monocytes [8] are recruited. These cells are followed by influx of inflammatory monocytes and eosinophils. Upon induction of the inflammatory response, the resident macrophage population is reduced and this cell population is replenished by proliferation when the inflammatory response is resolved [1,9].

Experimental peritonitis can be induced by a wide variety of stimuli such as various irritants, infection, particulate antigens and dead cells. The induction of sterile peritoneal inflammation by necrotic cells involves the stimulation of both resident macrophages and radioresistant cells [10] such as mesenchymal cells [11]. The necrotic cells will release cellular components known as damage-associated molecular patterns (DAMPs) [12,13]. It is well established that DAMPs can bind to and stimulate both TLRs and receptor of advanced glycation end products and thus induce a sterile inflammatory response. RAGE-deficient mice display reduced thioglycollate-induced peritonitis [14] and complementation of RAGE expression in endothelial cells reversed this phenotype. Stimulation of RAGE on endothelial cells induces VCAM-1 expression and promotes leukocyte transmigration [15–18]. Interestingly, peritonitis induced by necrotic cells is largely independent of TLRs [19]. Rather, the inflammatory response is mediated by uric acid released by necrotic cells [20] and involves the production of IL-1α and IL-1β [10]. IL-1 production in response to cellular necrosis involves activation of the NLRP3 inflammasome [21,22], which in turn can also be activated by monosodium urate crystals [23]. Alum adjuvant crystals can induce cell

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damage [24] thereby causing the release of uric acid [25]. It is known that alum adjuvant can induce IL-1β production in an NLRP3 inflammasome-dependent way [26]. However, alum can also stimulate innate immune mechanisms through NLRP3 inflammasome-independent mechanisms [27,28]. Because of the particulate nature of alum adjuvant, it will most likely also provide more extended immune stimulation as compared to necrotic cells. These two agents would therefore be expected to induce partially different inflammatory responses.

Our laboratory, as well as other investigators, has investigated the impact of quinoline-3-carboxamides (Q-compounds) on inflammatory conditions. These compounds have shown efficacy in several mouse models of inflammatory autoimmune disease [29–33] and they are currently in clinical development for multiple sclerosis [34–37], systemic sclerosis and prostate cancer [38,39]. Recently, the S100A9 protein was identified as one molecular target of the Q-compound paquinimod (ABR-215757) [40]. We have previously shown that paquinimod interferes with development of disease both in mouse models of multiple sclerosis [40,41] and systemic lupus erythematosus [42]. Further, our previous work indicated that this compound interfered with the accumulation of myeloid cells during inflammation [43]. Due to the efficacy of Q-compounds in several models of inflammatory disease, we reasoned that these compounds most likely target a mechanism common to these diseases. In here, we have therefore used sterile peritoneal inflammation as a model to determine whether paquinimod would interfere with the accumulation of inflammatory cells. Our results presented in this report indicate that this is indeed the case.

2. Materials and methods

2.1. Mice and treatment

Wild type C57Bl/6 mice were purchased from Taconic Europe (Ry, Denmark). All animal experiments were performed with the permit of the local committee on the ethics of animal experiment of Malmö and Lund (permits M4-11 and M12-13). To study the effects of the Q-compound paquinimod, mice at the age of 7–9 weeks were treated with paquinimod dissolved in drinking water at a concentration of 140 μg/ml (corresponding to a daily dose of about 25 mg/kg body weight/day) for 24 h prior to any other procedures. Paquinimod was provided by Active Biotech, Lund, Sweden.

2.2. Induction of peritonitis

EG7 cells (OVA-transfected EL4 lymphoma cell line) [44] were cultured in RPMI medium (RPMI-1640 supplemented with 10% fetal calf serum, 10 mM HEPES, 1 mM sodium pyruvate, 100 U/ml penicillin–streptomycin and 50 μM β-mercaptoethanol (all supplements from Invitrogen Life Technologies, Paisley, UK)) at 37 °C, 5% CO_2. The EG7 cells were obtained from Dr Clotilde Thery, Institute Curie, INSERM U932, Paris, France.

Necrosis was induced using the protocol from a previous study [19]. Briefly, the cells were harvested, washed twice with PBS (Invitrogen Life Technologies) and heat-shocked at 45 °C in water bath for 10 min and subsequently incubated at 37 °C for 4 h prior to use. Mice were injected intraperitoneally (i.p.) with 10^7 heat-shocked necrotic cells. Peritoneal cells were lavaged after 20 h by injection of 7 ml RPMI medium. The volume of recovered lavage solution was determined such that the total number of peritoneal cells could be calculated. Omenta were also collected. To prepare omental cells, we used the "walk-out" method previously reported by Carlow et al. [45]. In brief, omenta from similar cohorts of mice were pooled and placed in wells of flat-bottom 96-well plates in RPMI medium and incubated at 37 °C overnight. Cells migrating out from the omenta were collected and wells were washed with 10 mM EDTA (Millipore, Billerica, MA) to collect adherent cells.

Alternatively, mice were injected i.p. with 1 mg Imject alum (Thermo Scientific, Waltham, MA). In this setting, peritoneal cells and omenta were collected 4 h or 20 h after immunization. Peritoneal and omental cells were quantified using the Sysmex KX-21N automated hematology analyzer (Kobe, Japan).

2.3. Matrigel plugs

Growth factor-reduced matrigel, purchased from BD Biosciences (San Diego, CA), was injected subcutaneously (200 μl) in the flank. Matrigels were either substituted with PBS (3:1 vol/vol) or with PBS containing 1 mg Imject alum. Plugs were removed from mice 48 h later, cut into pieces with a scalpel and incubated on ice for 1 h in cell recovery solution (BD Biosciences). Finally, pieces were mashed through a 70 μm cell strainer. The cells obtained from matrigels were quantified using AccuCount beads (Spherotech, Lake Forest, IL).

2.4. Antibodies and flow cytometry

The following antibodies were purchased from Biolegend (Nordic Biosite, Täby, Sweden): CD11b-Alexa700, CD11c-APC-Cy7, F4/80-PE-Cy7, Ly6G-FITC and I-A/I-E (MHCII)-Pacific Blue. The following antibodies were purchased from BD Biosciences: CD19-PerCP-Cy5.5, Ly6C-biotin, streptavidin-BD Horizon V500 and SiglecF-PE. CD115-APC was purchased from eBioscience (Nordic Biosite, Täby, Sweden). Cells were stained with the above antibodies in FACS buffer (PBS supplemented with 5% fetal calf serum and 0.05% NaN_3 (Sigma-Aldrich, St. Louis, MO)). Propidium iodide (PI) (Invitrogen, Carlsbad, CA) was used to detect dead cells. Analysis of stained cells was performed using the LSRII flow cytometer (BD Biosciences).

2.5. Statistical analyses

Statistical analyses were performed using the Mann–Whitney U test.

3. Results

3.1. Paquinimod reduces accumulation of $CD11b^+$ cells during peritoneal inflammation

To study the impact of paquinimod on the recruitment of leukocytes to a site of inflammation, we used a mouse model of sterile peritoneal inflammation. The inflammation was elicited by injecting necrotic tumor cells [19] and this led to increased number of $CD11b^+$ myeloid cells in the peritoneal lavage obtained 20 h after immunization (Fig. 1B). Similar to observations in other peritonitis models [46], immunization with necrotic cells also caused the loss of resident $CD11b^+$ $F4/80^+$ peritoneal macrophages (Fig. 1A). The accumulation of $CD11b^+$ cells was significantly reduced in paquinimod-treated mice (Fig. 1B), suggesting that the compound might interfere with cell recruitment to the peritoneum during this inflammatory condition.

Peritoneal immunization with TLR agonists reduces peritoneal B cell numbers [47]. This involves CXCL13-dependent migration of B1 cells to the greater omentum [48] and may involve exit via efferent lymphatics [49], but paquinimod treatment had no effect on B cell numbers (Fig. 1C). In addition, it did not interfere with the immunization-induced loss of peritoneal DCs (Fig. 1C) and macrophages (Fig. 1A) either. Thus, while paquinimod treatment displayed significant effects on myeloid cell populations, it did not affect the dynamics of peritoneal B cells, DCs and macrophages. Taken together, these data indicate that paquinimod selectively affects cell dynamics in this model of peritoneal inflammation. Finally, paquinimod did not significantly influence the number of steady state peritoneal $CD11b^+$ cells in normal non-immunized mice (Fig. S1A), indicating that the compound does not have toxic effects on resident myeloid cells.