Journal of Immunological Methods 415 (2014) 36–45

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Murine antigen-induced inflammation—A model for studying induction, resolution and the adaptive phase of inflammation

Valgerdur Tomasdottir [a,b], Arnor Vikingsson [d], Ingibjorg Hardardottir [a,1], Jona Freysdottir [b,c,d,*,1]

[a] Department of Biochemistry and Molecular Biology, Faculty of Medicine, Biomedical Center, University of Iceland, Reykjavik, Iceland
[b] Department of Immunology, Faculty of Medicine, Biomedical Center, University of Iceland, Reykjavik, Iceland
[c] Department of Immunology, Landspitali - The National University Hospital of Iceland, Reykjavik, Iceland
[d] Center for Rheumatology Research, Landspitali - The National University Hospital of Iceland, Reykjavik, Iceland

ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form 27 August 2014
Accepted 22 September 2014
Available online 28 September 2014

Keywords:
Antigen-induced inflammation
Murine model
Induction of inflammation
Resolution of inflammation

ABSTRACT

Murine zymosan-induced peritonitis is the model most frequently used to study resolution of inflammation. However, the antigen-induced peritonitis model may be better suited for studying resolution of inflammation and the adaptive phase that follows. The objective of this study was to provide an evaluation of the kinetics of cells and mediators during induction, resolution and the adaptive immune phases of a murine antigen-induced inflammation.

Female C57BL/6 mice were immunized twice subcutaneously with mBSA and three weeks after the initial immunization they were injected intraperitoneally (i.p.) with mBSA, which induced peritonitis. Peritoneal cells were counted and expression of surface molecules and chemokine receptors analyzed with flow cytometry. Chemokine and cytokine concentrations in peritoneal fluid were determined by ELISA.

Two neutrophil populations, differing in size and granularity and slightly in expression of surface molecules, were observed in the peritoneal cavity after induction of inflammation. Macrophages disappeared from the peritoneal cavity following i.p. administration of mBSA but appeared again as they differentiated from recruited monocytes and peaked in numbers at 48 h. At that time point, two distinct populations of macrophages were present in the peritoneal cavity; one with high expression of F4/80, also expressing the atypical chemokine receptor D6 as well as CCR7; the other expressing low levels of F4/80 and also expressing CD11c and CD138. Eosinophils appeared in the peritoneum 3 h following i.p. administration of mBSA and peaked at 48 h. At that time point they had upregulated their expression of CCR3 but decreased their expression of CD11b. Peritoneal levels of CCL11 peaked at 6 h and may have led to recruitment of the eosinophils. NK cells and T cells peaked at 48 h, whereas B cells peaked at 5 days, with the majority being B1 cells. Peritoneal concentrations of pro-inflammatory cytokines (IL-β and IL-6) and chemokines (CCL2 and CCL3) peaked at 3 h, whereas IL-1ra peaked at 6 h, sTNF-R at 24 h and sIL-6R and TGF-β at 48 h.

The results show kinetic alterations in cell populations and mediators in a murine model that may be an excellent model to study initiation and resolution of inflammation and the following adaptive phase.

1. Introduction

The inflammatory response consists of an initial induction phase that evolves into a resolution phase. The fine tuning of these responses is essential for maximizing the

* Corresponding author at: Department of Immunology and Centre for Rheumatology Research, Landspitali - The National University Hospital of Iceland, IS-101 Reykjavik, Iceland. Tel.: +354 8612056; fax: +354 5434828.
E-mail address: jonaf@landspitali.is (J. Freysdottir).
[1] Joint last authorship.

http://dx.doi.org/10.1016/j.jim.2014.09.004
0022-1759/

benefits and minimizing the harm ensuing from activation of the inflammatory cascade. Although the inflammatory response is customarily initiated by activation of the innate immune system, adaptive immunity can serve a valuable role in diminishing inflammatory responses and accelerate recovery following re-exposure to an antigen.

Induction and resolution of inflammation has been thoroughly studied in the zymosan-induced peritonitis model (reviewed in (Serhan, 2007)), as well as in allergic airway inflammation (Haworth et al., 2011; Rogerio et al., 2012). Zymosan triggers the inflammatory response by binding to toll-like receptor (TLR)-2 on tissue monocytes/macrophages, thereby inducing cytokine and chemokine secretion. Marked but transient influx of neutrophils into the peritoneum is followed by infiltration and activation of monocytes/macrophages. Initially, the macrophages are pro-inflammatory (M1 macrophages), secreting tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 and vigorously phagocytosing foreign antigens and apoptotic neutrophils, but gradually their phenotype changes to an anti-inflammatory or pro-resolution type (M2 or rM macrophages, respectively) (Gilroy et al., 2004; Schif-Zuck et al., 2011), characterized by predominant production of pro-resolution lipid mediators (i.e., lipoxins, resolvins, protectins and maresins) and anti-inflammatory cytokines (e.g. IL-10 and transforming growth factor (TGF)-ß) (reviewed in (Ariel and Timor, 2013)). This resolution process may be further strengthened by the emergence of a recently described resolution-promoting eosinophils (Yamada et al., 2011). Eventually, the inflammatory lesion recedes and post inflammation homeostasis is restored.

Induction of inflammation within the sterile peritoneal cavity represents an ideal location to study the evolution of the inflammatory response due to the easy access to the peritoneal exudate for analysis of cells and soluble mediators. Therefore, zymosan-induced peritonitis is an excellent model to study an intense self-limited acute inflammation resulting from TLR activation. However, the zymosan-induced peritonitis model entails little or no activation of the adaptive immune response (Kolaczkowska et al., 2008) and may, therefore, not accurately reflect the kinetics of the induction and resolution phases during naturally occurring infections or during immune reactions that have the potential to evolve into chronic inflammation, including autoimmune processes. In the methylated BSA (mBSA)-induced peritonitis model, both the innate and the adaptive elements of the immune response are initially activated by immunizing subcutaneously (s.c.) with mBSA and complete Freund´s adjuvant. Peritonitis is subsequently induced by intraperitoneal (i.p.) injection of mBSA without an adjuvant, resulting in reactivation of the adaptive immune response. In previous studies, Cook et al. described temporal changes in the number of neutrophils and macrophages in mBSA-induced peritonitis (Cook et al., 2003, 2004) but only provided analysis of surface markers and soluble mediators within the peritoneal cavity on day 4 following induction of inflammation. In the present study, we provide a detailed analysis of cells and soluble mediators during the induction and resolution phases as well as the subsequent adaptive phase of inflammation in murine mBSA-induced peritonitis.

2. Materials and methods

2.1. Mice

All animal procedures were approved by the Experimental Animal Committee, Ministry for the Environment in Iceland (#0507-1503) and complied with NRC´s Guide for the Care and Use of Laboratory Animals. Female C57BL/6 mice, weighing 18–20 g, were obtained from Taconic Europe (Ejby, Denmark). They were housed 8 per cage with a 12 h light/dark cycle at 23–25 °C and 45–55% humidity. Mice were acclimated for 1 week prior to initiation of the experiments. Mice had free access to food and water.

2.2. Induction of peritonitis

mBSA (Sigma Aldrich, St. Louis, MO) was dissolved in water and 10× PBS added to give the final concentration of 1× PBS. Mice were immunized s.c. at the base of the tail with 100 μg of mBSA emulsified in equal volume of CFA (Sigma Aldrich) until white and thick. The total injection volume was 50 μl. Two weeks later, the mice were given a booster injection of 100 μg of mBSA in incomplete Freund's adjuvant (IFA) (Sigma Aldrich). Three weeks after the initial immunization, mice were challenged i.p. with 100 μg of mBSA in 50 μl of saline. Before and at several time points after peritonitis induction (3 h, 6 h, 12 h, 24 h, 48 h, 5 days and 10 days), mice were anesthetized with a mixture of hypnorm (VetaPharma Ltd, Leeds, UK), dormicum (Roche, Basel, Switzerland) and sterile water (1:1:2) and killed by cervical dislocation.

2.3. Collection of serum, peritoneal lavage and spleen

Serum was collected and stored at −70 °C. Peritoneal exudate was obtained by injecting 1.5 ml of cold phosphate-buffered saline (PBS), without calcium or magnesium, into the peritoneal cavity and collecting the fluid. Cells and supernatant were separated by centrifugation. The supernatant was collected and stored at −70 °C. Spleen was collected and pushed through a sieve in order to obtain single cell suspension. Red blood cells were lysed using ACK lysing buffer (0.15 M NH_4Cl, 1 mM $KHCO_3$ and 0.1 mM Na_2EDTA). Peritoneal and spleen cells were washed twice with PBS and resuspended in FACS buffer (PBS containing 1% BSA, 0.2 mM EDTA and 0.1% NaN_3) and counted by Countess automated cell counter (Invitrogen, Paisley, UK).

2.4. Characterization of cells by flow cytometry

Peritoneal and spleen cells (0.3×10^6) were incubated with 2% normal rat:normal mouse serum (1:1) (AbD Serotec, Kidlington, UK) for 20 min. Cells were stained with fluorochrome-labeled monoclonal antibodies (mabs) against Gr1, F4/80, CD115, CD90.2, NK1.1, CD4, CD8, B220, IgD, CD5 (eBioscience, San Diego, CA), Ly6G (clone 1A8), CD11b, F4/80 (BD Bioscience, San Jose, CA), CCR3, CXCR2, D6, CCR7 (R&D Systems, Abington, UK) and CD11c (MBL-Nordic Biosite, Taby, Sweden). Appropriate isotype controls were used to set the quadrants and evaluate background staining. Cells were washed twice, resuspended in FACS buffer, and 10,000 cells were collected on FACScalibur (BD Biosciences). Data were