Deciphering the peritoneal mononuclear system in mice: steady state and inflammation

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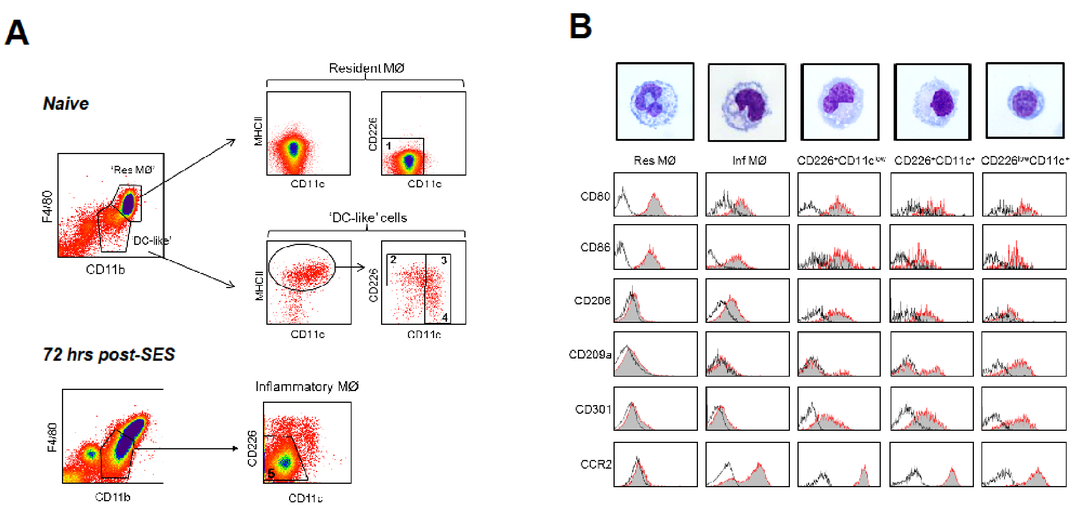
Objectives

Little is known about the peritoneal mononuclear phagocyte system. We aim to identify distinct mononuclear phagocyte subsets in the murine peritoneal cavity, and characterize their phenotypes, functions and kinetic changes during acute and chronic experimental peritonitis.

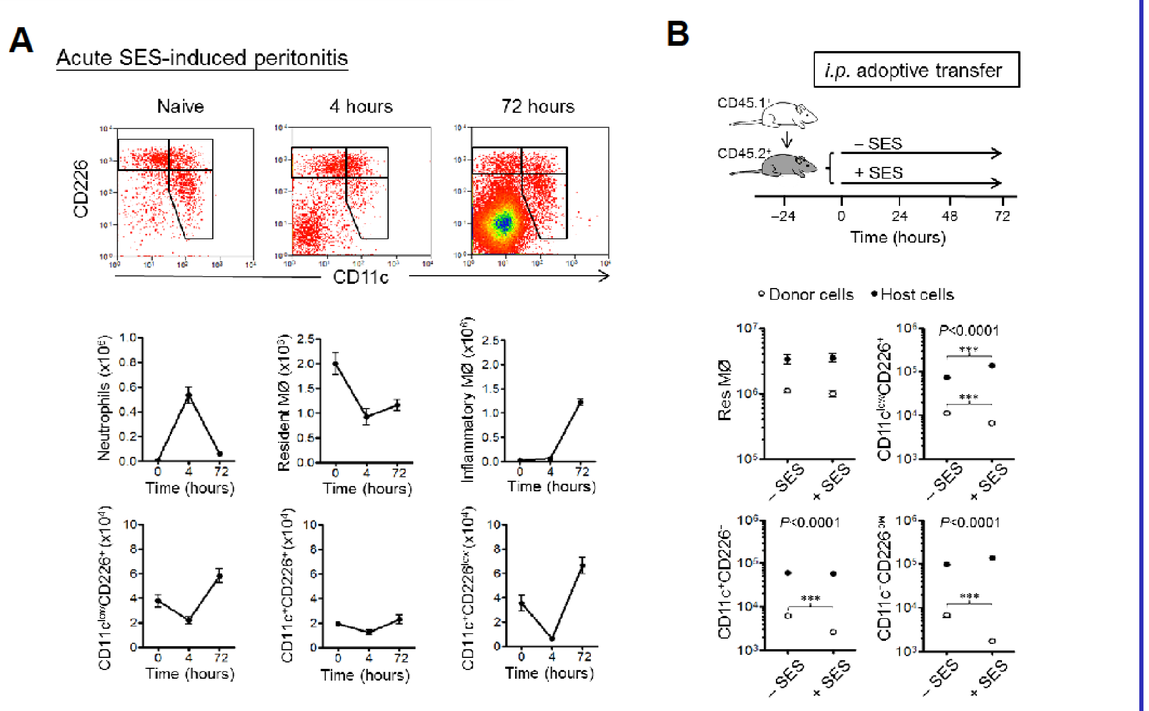
Results

1. Phenotypic identification of distinct peritoneal MØ/DC subsets



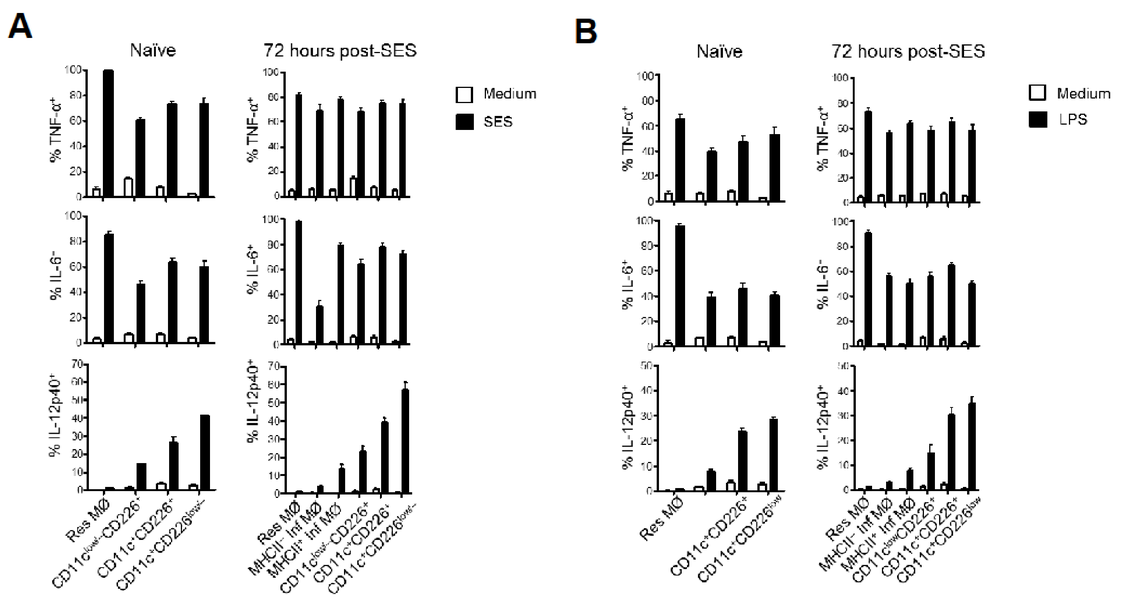
(A) Flow-cytometric analysis showing mouse peritoneal mononuclear phagocytes under naive status could be segregated into two main population, F4/80^{high}CD11b^{high}MHCII$^{low/-}$ resident macrophages (**1 : 'Res MØ'**) and CD11b^{int}F4/80$^{int/low}$MHCII^{high} **'DC-like'cells** (Dioszeghy et al. *J Immunol* 2008). The latter was subdivided into three subsets: **2: CD226^{+}CD11c^{low}, 3: CD11c^{+}CD226^{+}, 4: CD11c^{+}CD226^{low}**. At 72 hours post intraperitoneal (i.p.) *Staphyloccous epidermidis* supernatant (SES) challenge, a large number of **CD11c^{-}CD226^{-} inflammatory MØs** ('Inf MØ') accumulated within the peritoneal cavity. **(B)** Morphological features (cytospun cells stained with Hemacolor kit) and differential expression of selective surface markers (histograms) on these five distinct subsets of peritoneal mononuclear phagocytes were shown.

2. Cellular kinetics of different peritoneal MØ/DC subsets during acute SES-induced peritonitis



(A) Flow-cytometric analysis showing the kinetic changes of Inf MØ (CD11c^{-}CD226^{-}) and three 'DC-like' cells (CD226^{+}CD11c^{+}, CD226^{+}CD11c^{low}, CD11c^{+}CD226^{low}) during acute SES-induced peritoneal inflammation. Graphs showing the absolute numbers of neutrophils, Res MØ, Inf MØ and three DC subsets (as defined above) observed during acute SES-induced peritoneal inflammation. Data (mean ± SEM) are derived from 5- to 6- week-old C57BL/6 mice (n = 4 per group) and are derived from one of two independent experiments. **(B)** Schematic representation of the adoptive transfer strategy used to study to determine the origin of peritoneal 'DC-like' cells during peritonitis. One day after adoptive transfer of naïve peritoneal cells between CD45 allotype-mismatched animals, mice were either injected intraperitoneally with SES or were left unchallenged, and were sacrificed on Day 3. Graphs showing the number of recoverable donor (white symbols) and host (black symbols) Res MØ and 'DC-like' subsets in the peritoneal lavage of the mice. Data represents mean ± SEM. Data was analyzed for statistical significance by two-way ANOVA after log transformation and interaction statistics *P*-values and Bonfferoni post-tests (asterisks) are indicated where significant.

3. Differential cytokine responses among different peritoneal MØ/DC subsets upon *ex vivo* microbial product stimulation

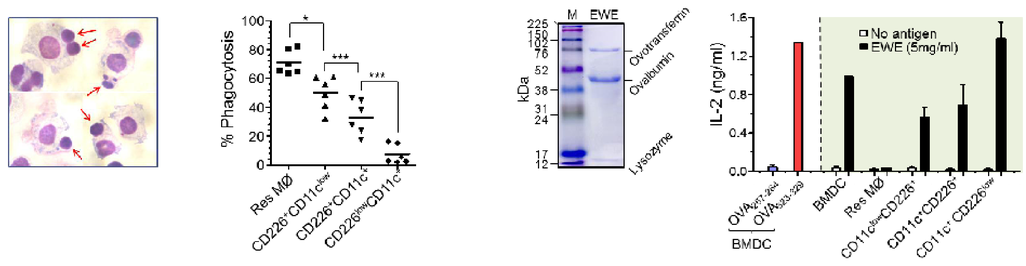


(A) Bar graphs comparing the intracellular cytokine production (TNF-α, IL-6, IL-12p40) after *ex vivo* stimulation with SES among individual MØ/DC subsets from C57BL/6 mice, both naïve (left panels) and 72 hours post-intraperitoneal SES challenge (right panels). Data represent mean ± SEM (n = 4-5 in each experiment). **(C)** Similar experiments with *ex vivo* LPS stimulation were undertaken. Data represent mean ± SEM (n = 4-5 in each experiment).

4. Distinctive functional specialization of peritoneal MØ/DC subsets

A Apoptotic thymocytes clearance

B Antigen processing and presentation



(A) Photos showing the cytospun peritoneal mononuclear phagocytes actively phagocytosing apoptotic thymocytes (red arrow). Graphs comparing the capability of apoptotic thymocytes clearance (% phagocytosis) among individual peritoneal MØ/DC subsets. Data represent the mean and are derived from one of two similar experiments (n = 5). **(B)** Coomassie stained SDS PAGE gel showing the protein content of EWE (5 µg resolved on a 12% SDS PAGE gel). Ovalbumin (OVA) (~45 KDa) is the major constituent of egg white. The ability of the purified cellular subsets from naïve C57BL/6 mice to process and present EWE on MHCII to BO-97.11 cells was assessed and compared to bone marrow-derived DC (BMDC) and BMDC pulsed with specific peptide. All three DC subsets were found to be competent at antigen processing and presentation. Data shows mean ± SEM of IL-2 production by the BO-97.11 T cell hybridoma from two independent experiments.

Conclusions

We have identifed multiple mononuclear phagocyte subsets in the murine peritoneal cavity. Functional specialization of individual MØ/DC subset and the respective inflammatory kinetics during acute peritonitis have been characterized. Our findings have paved the way for the development of novel therapeutic strategy, i.e., specific targeting inflammatory MØ/DC subset to ameliorate the repeated peritonitis induced peritoneal fibrosis.

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L2) Dialysis. Peritoneal dialysis.
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