Peritoneal macrophage heterogeneity is associated with different peritoneal dialysis outcomes

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Peritonitis remains the major obstacle for the maintenance of long-term peritoneal dialysis and dysregulated host peritoneal immune responses may compromise local anti-infectious defense, leading to treatment failure. Whilst, tissue mononuclear phagocytes, comprising macrophages and dendritic cells, are central to a host response to pathogens and the development of adaptive immune responses, they are poorly characterized in the human peritoneum. Combining flow cytometry with global transcriptome analysis, the phenotypic features and lineage identity of the major CD14$^+$ macrophage and CD1c$^+$ dendritic cell subsets in dialysis effluent were defined. Their functional specialization was reflected in cytokine generation, phagocytosis, and antigen processing/presentation. By analyzing acute bacterial peritonitis, stable (infection-free) and new-starter patients receiving peritoneal dialysis, we identified a skewed distribution of macrophage to dendritic cell subsets (increasing ratio) that associated with adverse peritonitis outcomes, history of multiple peritonitis episodes, and early catheter failure, respectively. Intriguingly, we also noted significant alterations of macrophage heterogeneity, indicative of different maturation and activation states that were associated with different peritoneal dialysis outcomes. Thus, our studies delineate peritoneal dendritic cells from macrophages within dialysate, and define cellular characteristics associated with peritoneal dialysis treatment failure. These are the first steps to unravelling the detrimental adaptive immune responses occurring as a consequence of peritonitis.

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Peritoneal dialysis (PD)-related peritonitis remains a major cause of technique failure as well as mortality in maintenance PD patients.[1,2] In PD patients, the dialysis exchange perturbs the tightly regulated tissue homeostasis and composition of the immune cell population within the peritoneal cavity.[3] Previously, we have delineated the local response to peritoneal infection–driven inflammation.[4,5] Our recent work has shown that repeated bacterial peritoneal inflammation drives immune-mediated compromised tissue repair response and fibrosis.[6] These data support the concept that alterations in peritoneal immune cell composition in PD patients may link to their adverse outcomes.

Tissue mononuclear phagocytes, comprised mainly of macrophages (MØ) and dendritic cells (DC), are a key tissue-resident component of the local immune system, with roles including pathogen clearance, tissue repair, and antigen presentation.[7,8] Recent progresses in MØ/DC biology have uncovered heterogeneity in these cell phenotypes, related to different developmental origins and distinct differentiation pathways.[9–11] Studies on the phenotypic and functional attributes of different tissue MØ/DC subsets *in vivo* have highlighted that distinct subsets have specialized roles in tissue homeostasis and local inflammation.[12,13] In the peritoneal cavity, studies have been largely focused on MØ in mice. We, and others, have recently identified a major, self-renewing population of tissue-resident macrophages, with roles in tissue homeostasis and response to inflammation.[14–16] On acute peritoneal inflammation induced by *Staphylococcus epidermidis* supernatant in mice,[17] neutrophils and monocytes are recruited into the inflamed peritoneum. These infiltrated monocytes will differentiate into MØ and/or DC and play effector functions locally (i.e., phagocytosis and apoptotic cell clearance, antigen presentation, and T-cell stimulation). In the field of PD, studies in late 1970s began to examine the cellular composition of dialysis effluent fluids from noninfected PD patients, and revealed the MØ as the predominant cell type found in dialysis effluent.[18,19] Based on *ex vivo* and *in vitro* functional analysis, peritoneal MØs are important in the front line of host peritoneal defense in PD patients.[20–23] It has been suggested that peritoneal MØs from PD patients phenotypically and functionally resemble *in vitro* polarized macrophage colony-stimulating factor–driven "anti-inflammatory" MØ or interleukin (IL)-4–driven alternatively activated MØ[24,25]; however, so far the comprehensive genetic profiling and related

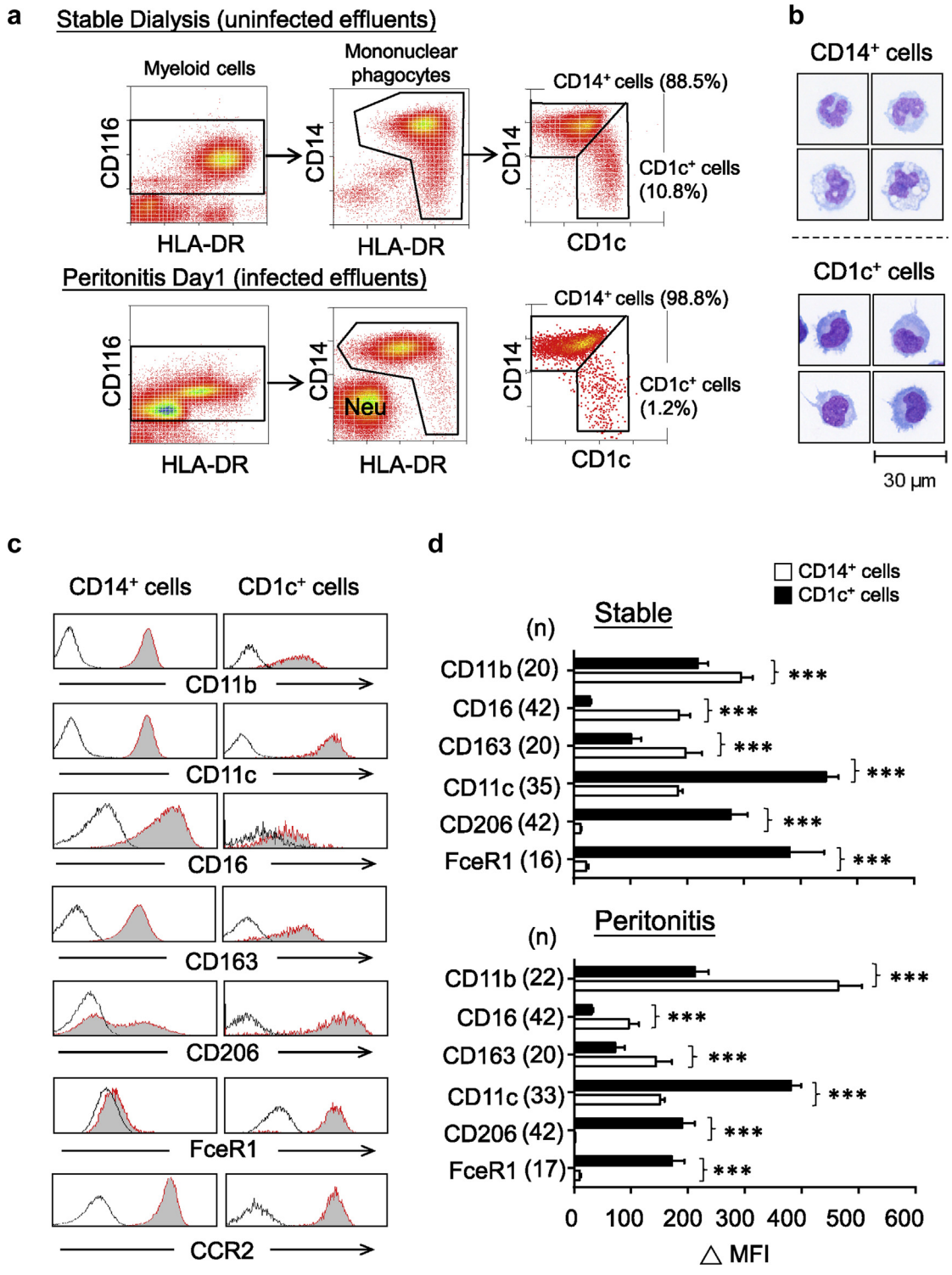

Figure 1 | Phenotypic identification of peritoneal mononuclear phagocyte subsets. (**a**) Representative density plots showing flow-cytometric gating strategies to identify peritoneal mononuclear phagocyte subsets within PD effluent from stable dialysis patients (upper panel) and day 1 peritonitis patients (lower panel). Myeloid cells were pregated on $CD116^{+}$ populations after exclusion of doublets, cellular debris, and dead cells. Within these cells, mononuclear phagocytes could be readily identified as $HLA\text{-}DR^{+}CD14^{+/-}$, and granulocytes were $HLA\text{-}DR^{-}CD14^{-}$. The latter mainly comprised $CD16^{high}$ neutrophils (Neu), which substantially increased in number during acute peritonitis. Mononuclear phagocytes could be segregated into 2 subsets: $CD14^{+}CD1c^{low/-}$ (major) and $CD1c^{+}CD14^{low/-}$ (minor). (**b**) Sorted $CD14^{+}$ cells and $CD1c^{+}$ cells (the purity >95%) were cytospun, air-dried, and stained with Microscopy Hemacolor. The morphology of cells is shown (bar = 30 μm). Data derived from 1 patient representative of 4 stable patients. (**c**) Flow-cytometric analysis of select marker expression by $CD14^{+}$ cells and $CD1c^{+}$ cells. Representative histogram plots are pregated as described previously. Shaded histograms depict (Continued)