Settling in for the Long Term – Alternative Life Styles for Inflammatory Monocytes?

It is generally accepted that monocytes recruited from the bloodstream play a major part in inflammation. However, the ultimate fate of these elicited cells is much less clear and in particular, it has been controversial whether recruited monocytes can subsequently differentiate into resident macrophages with homeostatic properties. In the current issue of *Nature Immunology,* Gundra and colleagues[1] present evidence that monocytes recruited to the peritoneal cavity by a type 2 inflammatory reaction can differentiate into macrophages with the phenotypic and transcriptional signature of resident peritoneal macrophages. This process takes some weeks after the initial inflammatory insult and is dependent on vitamin A, a factor shown previously to be essential for the specification of resident peritoneal macrophages under steady state conditions[2]. Gundra *et al*. suggest a similar vitamin A driven process occurs in Th2 dependent granulomata that develop during infection of the liver with *Schistosoma mansoni*.

Macrophages play vital homeostatic roles such as providing trophic factors for tissue cells, clearance of effete cells and tissue remodelling, but are also crucial components of inflammatory reactions, contributing as effector cells to microbial defence and pathology, as well as being involved in resolution and tissue repair[3]. As it is now clear that the macrophage lineage is highly heterogeneous, there is considerable interest in determining whether these disparate functions are fulfilled by the same cells, or if separate populations are required. As a first step to exploring this issue in the peritoneal cavity, Gundra et al induced the local recruitment of monocytes by ip injection of thioglycollate, together with immune complexes consisting of IL4 + anti-IL4 antibody, a formulation that leads to sustained release of IL4 *in vivo*[4]. This protocol ablates the resident F4/80^{hi}MHCII^{-}

macrophage pool and generates a population of F4/80^{int}CD11b^{+} inflammatory monocytes that belong to the "alternatively activated" lineage of macrophages (referred to here as AAM^{mono})[5]. Some of these AAM^{mono} persisted in the cavity for several weeks after inflammation had resolved, during which time they progressively downregulated signature AMM^{mono} markers such as PD-L2 and then CD206, before acquiring the F4/80^{hi}MHCII$^{–}$ CD206$^{–}$PD-L2$^{–}$ phenotype that characterises alternatively activated resident peritoneal macrophages (AAM^{res}) that have been exposed to IL4 *in vivo*. The alternatively activated macrophages that are derived from converted monocytes (AAM^{conv}) also acquired other characteristics typical of their resident macrophage counterparts, including expression of the mitochondrial thermogenic protein UCP1 and *in situ* proliferative activity. The authors went on to confirm directly that the AAM^{conv} were the descendants of AAM^{mono} using a genetic fate mapping approach, in which tamoxifen-inducible Cre recombinase is under control of the *Cx3cr1* promoter, allowing the fate of CX3CR1^{+} monocytes/macrophages to be tracked over time. This system is particularly useful in this context because CX3CR1 is usually not expressed by resident macrophages in the peritoneal cavity[6] and it confirmed the ability of IL4 + thioglycollate-elicited monocytes to convert into F4/80^{hi} AAM^{conv} in the peritoneum over a period of weeks. In the final experimental approach, AAM^{mono} were adoptively transferred into the peritoneum of resting mice, again resulting in the appearance of AAM^{conv} with the appearance of AAM^{res}. RNA-sequence analysis revealed substantial overlap between AAM^{conv} and AAM^{res} at the transcriptional level, with both populations being very different from the starting population of AAM^{mono}. This corresponded with broadly similar landscapes of accessible chromatin in the resident and converted AAM as shown by the ATAC-seq assay (Assay for Transposase-Accessible Chromatin), with accessibility of the AAM^{res} signature gene *Ucp1* being one of the shared

features. Interestingly and despite the fact that IL4 was used to generate the AAM^{mono}, their subsequent conversion into resident-type macrophages was independent of IL4, and of the STAT6 and IRF4 transcription factors associated with type 2 immune responses. However the development of AAM^{conv} was entirely dependent on the presence of vitamin A in the diet.

In parallel experiments, again using the CX3CR1-based fate-mapping systems, Gundra *et al.* tracked monocyte fate in the context of the Th2 dependent response that drives chronic granuloma formation in the liver after *S mansoni* infection. Under these conditions, elicited monocytes also showed vitamin A dependent conversion into macrophages that acquired UCP1 and showed evidence of clonal proliferation. The absence of vitamin A disrupted formation of mature granulomata and the infected mice died more rapidly, although a direct link between these outcomes and monocyte conversion was not demonstrated.

The role of monocytes in maintaining tissue resident macrophage populations has been controversial, particularly after a number of studies proposed that these cells were derived from self-renewing embryonic precursors[3]. Nevertheless it is now clear that resident macrophages in the intestine require monocyte replenishment throughout adult life[7] and there is also increasing reliance on monocytes in other tissues such as the dermis, heart, lung and even the peritoneal cavity as animals age[8-11]. Furthermore recent work suggests that elicited monocytes can restore the resident macrophage pool of the liver if this niche is depleted without an inflammatory insult[12]. Despite this emerging evidence that monocytes can generate resident macrophages in steady state tissues, there has been a consensus that most if not all recruited monocytes cannot do this under inflammatory conditions[3].

The work of Gundra and colleagues thus provides new insights by indicating that monocytes elicited under type 2 immune conditions can persist and eventually acquire many of the characteristics of resident F4/80^{hi} peritoneal macrophages. Importantly, this conversion is driven by one of the factors that controls the homeostatic maintenance of resident peritoneal macrophages and overall, the results are further support for the idea that tissue environment rather than origin determines macrophage specification[12,13]. The findings also raise the prospect that it may be possible to restore tissue homeostasis by eg transfer of naïve monocytes under conditions in which resident macrophages have been depleted or compromised by inflammatory insults, such as in fibrotic disease, or chronic inflammation.

A number of issues would be need to be addressed before such ideas could be put into practice. First, it would be important to know how generalizable the findings of Gundra et al using a highly type 2 polarised model of inflammation might be to other tissues and forms of inflammation. That this may indeed be the case is suggested by previous findings that thioglycollate-elicited monocyte-derived macrophages can persist in the peritoneal cavity in the absence of IL4 for up to 8 weeks[6]. Nevertheless the current experiments did not address whether host gender had an influence on monocyte fate, as has been shown recently in the steady state peritoneal cavity[11]. The precise role of vitamin A in determining monocyte fate also needs to be elucidated. As in other studies, Gundra et al interpreted the failure to generate resident macrophages in mice on a vitamin A deficient diet as indicating an intrinsic role for retinoic acid in monocyte-macrophage development. However vitamin A deficiency has many effects on the animal, not the least being that it can lead to the development of inflammation in several tissues, including the peritoneum[14]. Therefore

altered monocyte fate in these mice could be secondary to more generalised dysregulation of immune homeostasis.

A further area for clarification will be the efficiency of differentiation by elicited inflammatory monocytes. As shown here, only a small proportion of the original monocyte population eventually acquires the characteristics of resident macrophages and it may be that this fate is a rare outcome of monocyte differentiation, or that only a restricted proportion of monocytes possess the capacity to become resident macrophages. Interestingly, monocyte persistence appeared to be particularly compromised in the liver granuloma model, perhaps suggesting an important role for niche availability in regulating monocyte fate, as the resident macrophage pool may have been depleted to a lesser degree by *S mansoni* infection than by thioglycollate. Even more important is how complete the functional overlap between converted monocytes and resident macrophages may be. Gundra *et al.*'s finding that over 1700 genes remained differentially expressed between these two populations in the peritoneal cavity contrasts with recent work in the liver, where there was almost complete transcriptional identity between monocyte-derived and tissue-resident Kupffer cells when these had been partially depleted in the absence of inflammation. As a first step, it would be interesting to assess the ability of converted monocytes to express markers characteristic of resident peritoneal macrophages, such as GATA6 and CD102. In parallel the question arises of how stable the conversion processes are over extended time periods. The epigenetic studies carried out by Gundra et al indicated that chromatin accessibility in converted monocytes was already similar to resident macrophages by 8 weeks after conversion. However more detailed ChIP sequencing experiments will be needed to determine whether plasticity is now permanently precluded

and so whether this approach might provide a long term means of modifying tissue homeostasis.

Legend to Figure

Inflammatory monocyte fate and macrophage differentiation in the peritoneum. In the steady state peritoneal cavity, most resident macrophages are F4/80^{hi}MHCII^{lo}CD206^{-} macrophages and there are a few F4/80^{lo}MHCII^{+} macrophages. F4/80^{hi} resident macrophages self-renew through *in situ* proliferation and rely on the transcription factor GATA6 for their maintenance (Ref.2). F4/80^{hi} macrophages were thought to derive exclusively from embryonic precursors (orange cells). However recent work suggests that F4/80^{lo}MHCII^{+}CD206^{+} macrophages that are derived from continuous replenishment by Ly6C^{hi} monocytes can mature over time into F4/80^{hi} macrophages (grey cells) in a sex-dependent manner (Ref.11).

Upon administration of thioglycollate and IL4c, the F4/80^{hi} 'resident' macrophage compartment is ablated and monocyte-derived, F4/80^{lo}MHCII^{+}CD206^{+}PD-L2^{+} macrophages (AAM^{mono}) come to dominate the peritoneal cavity.

As inflammation resolves, the AAM^{mono} compartment contracts in number, but some persist and under the influence of dietary vitamin A, convert into long-lived F4/80^{hi} resident macrophages (AAM^{conv}). This is a stepwise process involving downregulation of signature markers of AAM^{mono}, such as PD-L2 and CD206, and upregulation of UCP-1, a characteristic feature of IL4-experienced F4/80^{hi} resident macrophages (AMM^{res}).

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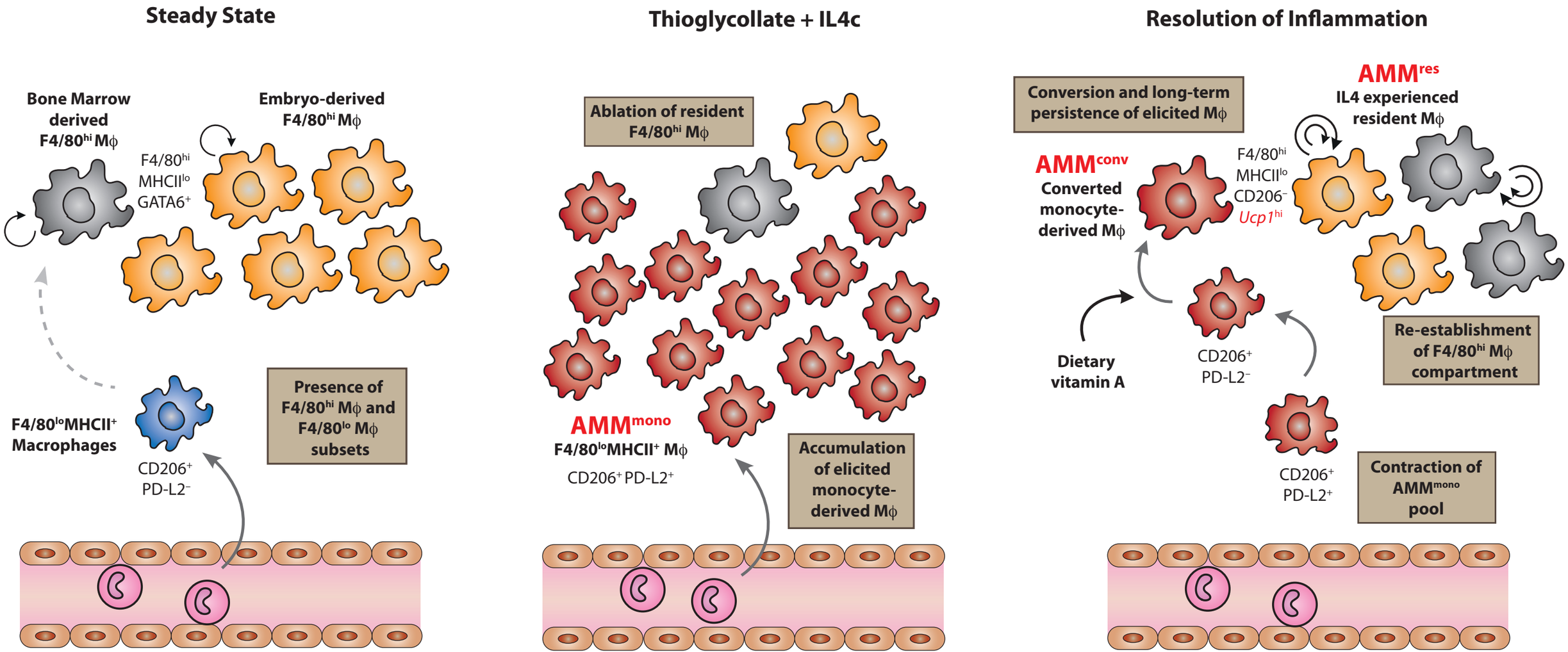
Steady State
Bone Marrow derived F4/80hi Mϕ
Embryo-derived F4/80hi Mϕ
F4/80hi MHCIIlo GATA6+
F4/80loMHCII+ Macrophages
CD206+ PD-L2−
Presence of F4/80hi Mϕ and F4/80lo Mϕ subsets
Thioglycollate + IL4c
Ablation of resident F4/80hi Mϕ
AMMmono
F4/80loMHCII+ Mϕ
CD206+ PD-L2+
Accumulation of elicited monocyte-derived Mϕ
Resolution of Inflammation
Conversion and long-term persistence of elicited Mϕ
AMMconv
Converted monocyte-derived Mϕ
AMMres
IL4 experienced resident Mϕ
F4/80hi MHCIIlo CD206− Ucp1hi
Dietary vitamin A
CD206+ PD-L2−
CD206+ PD-L2+
Re-establishment of F4/80hi Mϕ compartment
Contraction of AMMmono pool