The skills of immigrant plumbers

The bodies of animals, in marked contrast to those of plants and fungi, contain large numbers of cells that can move, and animal development depends on cell migration at a variety of scales. Most of your face, for example, came from the back of your head. The cells that give rise to your eggs or sperm lived in a safe place outside your body when it was arranging its basic anatomy, and entered it to migrate along your primitive gut until they got close to your developing gonads, which they then invaded (those developing gonads were well inside your body at that stage, even if you are male). Even an adult human body contains millions of immune cells that are free to patrol its tissues and react to and destroy any invading microbes. Those immune system cells include several types, one of which was identified by Élie Metchnikoff in 1884. Metchnikoff noted the ability of these cells to swallow up and destroy microbes and cell debris and he called them ‘macrophages’ - literally ‘big eaters’.

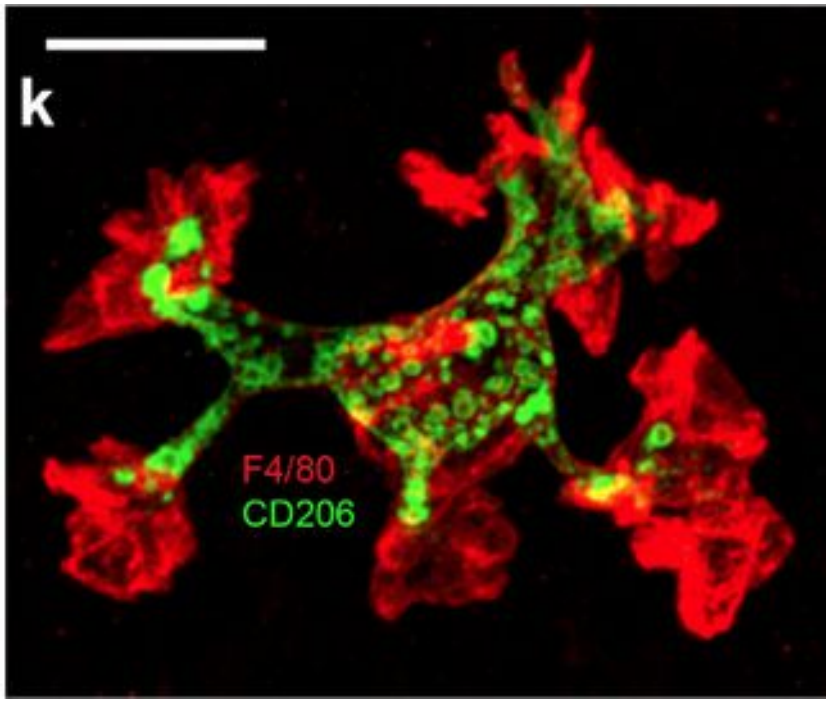


Figure 1: portrait of a macrophage (an image taken from the paper discussed in this blog article). The two colours are stains for two molecules associated with different parts of the macrophage cell (but not with other cell types). The horizontal bar is there to indicate scale, and is 0.01mm long (ie 10µm).

Macrophages were for many years considered only as defensive cells; ‘professional phagocytes’ that destroy both microbes and life-expired defensive cells of other types. It was later realized that they are important in teaching the immune system to recognize microbes, by presenting fragments of those microbes to cells of the adaptive immune system. Later still, researchers realized that

macrophages could have a constructive role as well as a destructive ones. Following muscle damage, for example, there are two waves of macrophage invasion, the first containing macrophages that eat up debris and the second containing macrophages that do not do anything destructive but instead secrete signalling molecules that promote muscle regeneration and also contribute directly to making matrix molecules to help the muscle cells. There is still lively discussion about whether these macrophages are really of different types, or are two lifestyles that can be adopted by the same basic kind of cell, depending on circumstance.

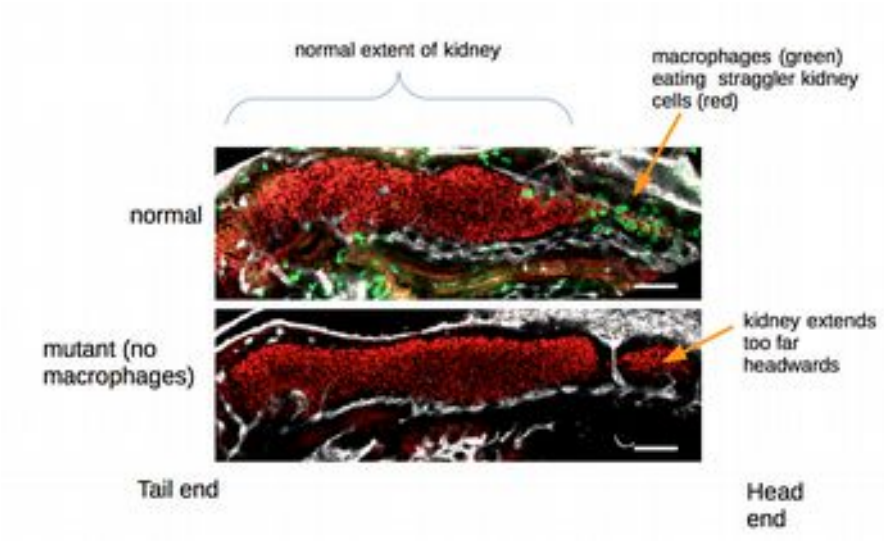
Given the varied roles of macrophages in adult life, it was natural that embryologists should look for them in early development. They saw macrophages enter the tissues of the body in distinct waves. The first wave comes from outside the body proper, from an extra-embryonic membrane called the yolk sac. Some of these settle in the liver and then give rise to a second wave, and a third wave comes from a completely different source, the primitive blood-forming system of the body (adult macrophages also come from the blood-forming system, which moves to the bone marrow). It has been known for some time that macrophages enter the developing kidneys from even the first of these waves as well as later ones, but it has not been clear what they do there. This question – what do macrophages do in the developing kidney – intrigued David Munro, a PhD student in my lab whose work has featured in two of these blogs already. The results of his research, summarized here, have just been published in full detail in the journal eLife (see Links section): I should make clear that, while I call this ‘David’s paper’ for reasons of conciseness in this blog, it was written with help from various experts in macrophages and molecular analysis methods and has ten authors in all. One of his co-authors is Julia Tarnick, another PhD student in this lab, some others are Edinburgh people (Chris Vink, Zhaun Li, Elaine Dzierzak), while yet others are from further afield (our old friend Peter Hohenstein, now in the Netherlands, is there, as are our new collaborators Yishay Wineberg and Tomer Kalisky from Israel).

It turns out that macrophages do more than one job (and David has probably identified only some of their roles: I would guess that much remains to be discovered). The first job is related to macrophages ability to clear things up, and in a very real sense these cells are needed to define exactly where the kidney begins and ends. To anyone used to adult anatomy, that may be a strange sentence as kidneys are clearly bounded by a fibrous capsule and ‘float’ relatively freely in the body, but early in development the kidney is just a zone within a continuous block of cells (the intermediate mesoderm) and it forms its boundary capsule only later. Cells that will form a kidney

can be identified by their expression of genes such as Six2 (in mouse). Initially, the boundaries of the kidney are somewhat ragged and Six2-positive cells spread out head-wards beyond what will be the zone of the kidney. Then, David found, a bunch of macrophages arrive and swallow up these straggler kidney cells. In mutant mice that cannot make macrophages, the straggler kidney cells remain and the kidney is longer than normal (Fig 1) and its development is delayed.

Later in kidney development, blood vessels invade the young organ and make its vascular system (one of David's earlier papers described this invasion in unprecedented detail: see the blog article 'bloody cyclists'). It turns out that, as the blood vessels enter, macrophages travel with them, not inside where the blood would be but outside the vessels, mostly making contact with the walls of the smallest vessels. Inhibiting vascular development using drugs resulted in reduced macrophage invasion, suggesting that the macrophages are following and serving the vessels rather than present independently of them.

Figure 2: macrophages normally restrict the kidney-forming area of the body by eating up out-of-place straggler kidney-forming cells that extend too far headwards. In a mutant that cannot make macrophages, these cells persist. The images are from David's paper, but the labels have been added for this blog.



What were the macrophages doing? A hint came from David's observation that the remains of blood vessel walls and blood cells could sometimes be seen inside macrophages, suggesting they were engaged in eating vessels. This apparently destructive activity might seem at odds with development but, given the location of the macrophages, it may well be that they promote the development of blood systems by promoting connection between small vessels, something that would generate debris as vessel walls open up to cross-connect. To test this, David inhibited

macrophage function and compared the architecture of the blood systems in kidneys with or without normal macrophage function (all of this was done in culture, not in living animals, by the way). Without active macrophages, kidneys still showed vessel growth but many of the vessels were isolated instead of being connected to a proper system. This implies strongly that the macrophages are important in connecting developing blood vessels together.

There is much more in the paper, but for me the biggest take-home message is that the development of the kidney's complicated blood system is not something its vessels can do for themselves. Instead, they require the services of macrophage 'plumbers', who migrate in to the organ with the vessels and are therefore in exactly the right places to wield their molecular hack-saws and mole-wrenches to make connections just where they are needed.

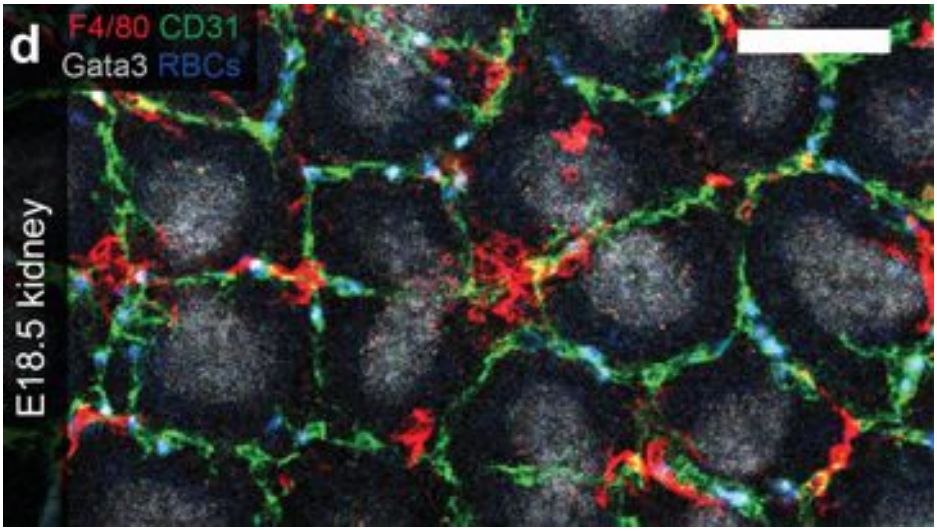


Figure 3: An illustration of how closely macrophages chaperone small blood vessels. The vessels are stained green, and the macrophages red. The blue stain shows blood cells inside the vessels, while the grey shows the tubes of what will be the urine collecting duct system, around which vessels develop (see the earlier blog 'bloody cyclists').

David now has his PhD and is getting used to being 'Dr. Munro', and to beginning a post-doctoral fellowship in which he will combine the skills he has learned so far with new ones in the fields of endocrinology and metabolism. I look forward to reading of his future work.

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Links:

- David's paper: https://elifesciences.org/articles/43271