Metabolic immunomodulation of macrophage functional plasticity in nonhealing wounds

Catherine B. Anders, Tyler M.W. Lawton, and Mary Cloud B. Ammons

Purpose of review
Despite modern advances in medicine, nonhealing wounds are the number one cause of nontraumatic, lower-limb amputation. Nonhealing wounds are characterized by a healing process stalled between inflammation and tissue remodel/repair, a stage characterized by a shift in macrophage functional phenotype. Characterization of diversity in macrophage functional phenotype in wounds and metabolic contributions to macrophage polarization are discussed.

Recent findings
Macrophage functional diversity in phenotype has recently evolved from duality (classically activated, pro-inflammatory M1 and alternatively activated, anti-inflammatory M2) to include an additional four alternately activated subphenotypes (M2a, M2b, M2c and M2d). Metabolic pathway utilization shifts characterize macrophage polarization with resulting metabolic and immune outcomes impacting host–pathogen interactions during wound healing.

Summary
Recognition of the key role macrophage diversity plays in wound healing, along with better characterization of diverse macrophage phenotypes, will inform our understanding of pathogenicity in wound healing. Comprehensive profiling of the metabolism regulating macrophage polarization and host–pathogen interaction creates opportunity of discovery for innovative new diagnostics and therapeutics for treating nonhealing wounds.

Keywords
immunomodulation, inflammation, macrophage, metabolism, wounds

INTRODUCTION

Over the past decade, significant progress has been made in the treatment of acute injuries. However, nonhealing in chronic wounds such as diabetic foot ulcers (DFUs), venous insufficiency ulcers (VIUs), pressure ulcers and unresolved hospital-acquired infections (HAIs) remains in an upward trajectory, despite the introduction of innovative therapeutics [1]. Such chronic wounds are defined as lasting greater than 30 days and are characterized by a failure to progress through the normal wound healing process [2]. In chronic wounds, the healing process appears stalled at the resolution of inflammation and initiation of tissue reorganization and this transition from early to late-stage inflammation is characterized by a shift in population from neutrophils to macrophages [3]. Under conditions of metabolic dysregulation, a chronic inflammatory state of tissue-resident macrophages is found in insulin-sensitive tissues such as adipose tissue [4], liver [5] and muscle [6]; however, the contribution of metabolism to macrophage functionality at the site of bacterial colonization in the wound has only recently started to be explored. Herein, the recently expanding role of macrophage functional phenotype and plasticity within the wound environment is discussed, including key findings that indicate this plasticity is achieved through metabolic immunomodulation. Viewed through the lens of infectious diseases, metabolism impact on macrophage functionality must play an essential role in pathogenesis, including the impact of metabolite exchange on host–pathogen interactions.

Idaho Veterans Research & Education Foundation (IVREF) – Boise VA Medical Center (BVAMC), Boise, Idaho, USA

Correspondence to Mary Cloud B. Ammons, Mail Stop 151, Building 117, 500 West Fort Street, Boise, ID 83702, USA. Tel: +1 208 422 1219; e-mail: MaryCloud.AmmonsAnderson@va.gov; mcammons@gmail.com

Curr Opin Infect Dis 2019, 32:204–209

DOI:10.1097/QCO.0000000000000550

KEY POINTS

- Chronic wounds are characterized by the healing process stalling at the transition between inflammation and tissue repair/remodelling.
- Macrophage functional diversity and plasticity in the changing environment makes these innate immune cells essential mediators of wound progression through the normal wound healing process.
- Metabolic immunomodulation of macrophage functional phenotype is key to innate immune response to wounding and host–pathogen metabolic interaction may be a key determinate of whether a wound resolves or stalls in the healing process.

PLASTICITY IN MACROPHAGE FUNCTIONAL PHENOTYPE

Although the earliest reports in macrophage literature focused primarily on two distinct phenotypes (termed classically and alternatively activated) [7], emerging research has identified several additional functional macrophage phenotypes that regulate immunological responses [8]. The commonly employed M1-M2 nomenclature (pro-inflammatory vs. anti-inflammatory, respectively) has expanded to include subphenotypes within the broader 'alternately-activated' macrophage classification (M2) indicated by the addition of lower case letters (i.e. M2a, M2b, M2c and M2d) [9]. Emergent findings attempting to characterize and define each of these distinct macrophage functional phenotypes clearly suggest that macrophage plasticity is essential to all stages of wound healing, as outlined in Fig. 1.

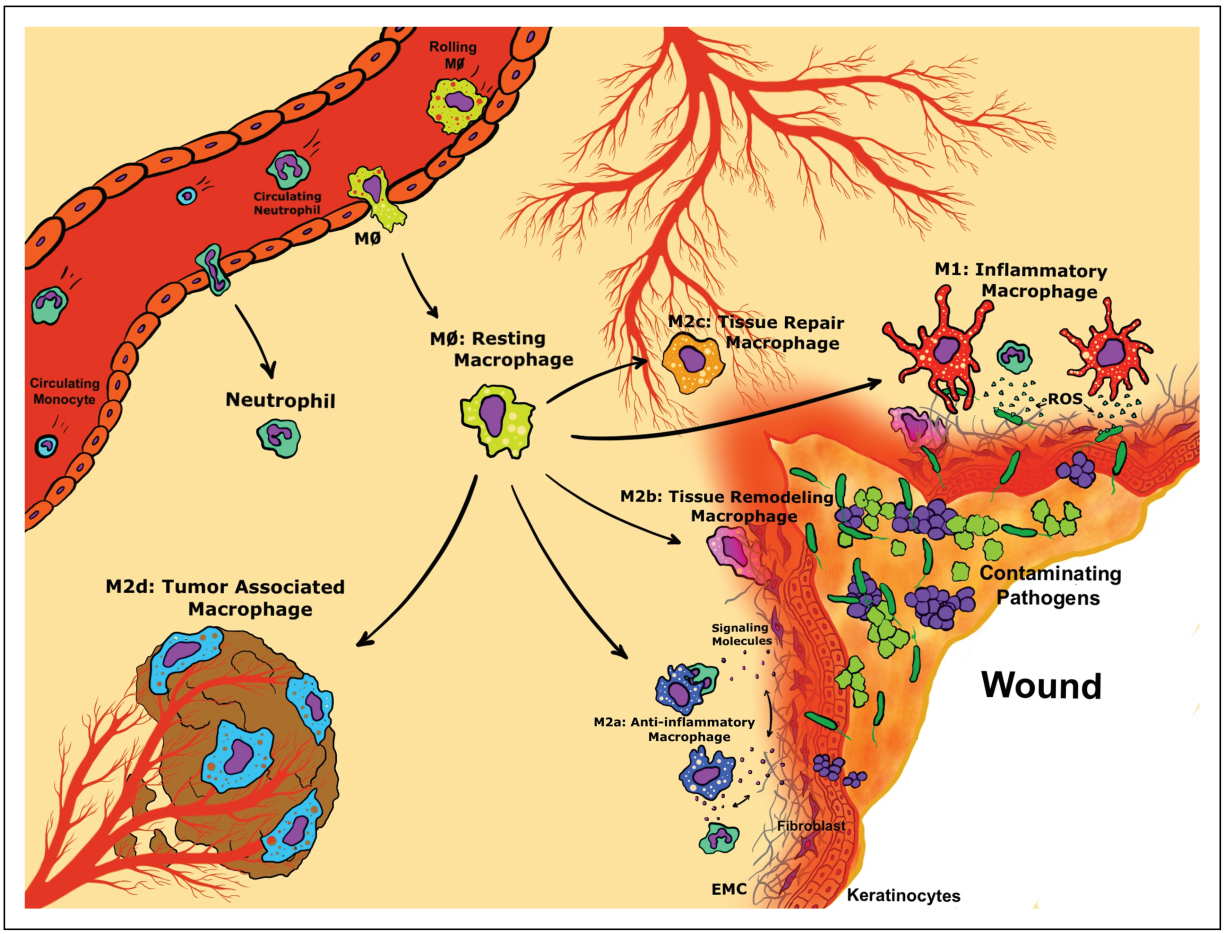

FIGURE 1. Macrophage phenotype functional diversity in the wound-healing environment. Graphical representation of macrophage functional phenotypes follow normal wound healing progression from postinjury recruitment of circulating innate immune cells to localized macrophage differentiation (M0: Resting Macrophage, yellow cells). Activation of macrophage polarization may result in one of five distinct functional phenotypes as follows: M1 inflammatory macrophages (red cells), M2a anti-inflammatory macrophages (dark blue cells), M2b tissue remodelling macrophages (purple cells), M2c tissue repair macrophages (orange cells) or M2d tumour associated macrophages (light blue cells). Original art by coauthor Tyler Lawton.

Upon signalling of tissue damage and bacterial colonization, resting M0 macrophages (yellow cells, Fig. 1) are activated from tissue-resident macrophages, recruited from circulating macrophages or differentiated from circulating monocytes that traffic to the site of injury. Although in a homeostatic metabolic state, the M0 macrophages represent the initiating step towards macrophage activation into the following variety of functional phenotypes. Most well characterized, the M1 or classically activated macrophage (red cells, Fig. 1) initiates the pro-inflammatory immune responses to pathogen colonization [10,11]. Recent advances describing M1 polarization suggest activation of circulating macrophages to this phenotype (M0 to M1) results in development of systemic inflammatory response syndrome in humans [12▪], demonstrating the lethality of inappropriate M1 sustained activation. As the inflammation abates, alarmins are believed to facilitate the onset of anti-inflammatory or wound healing responses characterized by the presence of alternatively activated macrophages or the M2 phenotypes.

Previously observed in fungal, parasite and helminth infections, the M2a phenotype is known to antagonize pro-inflammatory responses (dark blue cells, Fig. 1) and is observed in the tissue environment promoting wound resolution [13]. Distinct to the M2a phenotype, the mannose receptor (CD206) is thought to play a role in the elimination of pro-inflammatory proteins in wounds, but recent work using partially depleted CD206-M2a macrophages suggest these cells may also play a role in adipose tissue browning and insulin sensitivity [14▪▪]. A hybrid between the well described M1 and M2a phenotypes, the M2b macrophages (purple cells, Fig. 1) secrete both pro-inflammatory and anti-inflammatory cytokines/chemokines, and are thought to modulate the breadth and depth of an inflammatory response, blunting the immune response to infection, specifically in cases of infection postburning [15], without complete suppression of the inflammatory response as initiated at wounding.

Resolution of inflammation and transition to tissue formation is thought to be mediated by the M2c phenotype (orange cells, Fig. 1). M2c polarized macrophages demonstrate strong phagocytic activity targeted at uptake of apoptotic neutrophils and are thought to appropriately control wound repair response and limit collagen deposition in scar tissue [16]. Recent findings by Lurier *et al.* [17] demonstrated that the M2c phenotype upregulated several genes and cell-markers instrumental in normal wound healing through regulation of clot formation and angiogenesis, phagocytosis of wound debris and the deposition of ECM components. Although M2a and M2c functional phenotype seem to overlap, temporal activation of these two phenotypes occur separately in the process of normal wound healing with M2c gene expression peaking around 6 h postinjury and M2a gene expression peaking around 25 days postinjury [17,18].

Finally, tumour-associated macrophages (TAMs) or M2d macrophages (light blue cells, Fig. 1), have been observed within the tumour mass microenvironment, as implicated by the commonly used nomenclature. This phenotype is associated with potent immunosuppressive functions and angiogenesis promotion contributing to the survival of the tumour mass [19▪▪] and is thought to arise via tumour-secreted factors [20]. Although typically observed associated with a tumour mass, M2d macrophages have recently emerged as an important functional phenotype in the chronic wound environment, possibly through the suppression of T-cell immunity [21▪]. Chronically inflamed wounds are oxygen restricted, leading to miRNA epigenetic modification of hypoxia-related genes, which drives the phenotypic shift from M1 to M2d and contributes to M2d functionality [22▪▪,23]. Much debate remains about the identification and functional of wound-associated macrophage subpopulations, including contribution to healing in wounds (reviewed in Wermuth and Jimenez [24]); however, metabolic immunomodulation of macrophage plasticity in infectious disease is one of the most innovative area of research and has revived interest in these overlooked immune cells in pathogenesis.

METABOLISM AND MACROPHAGE FUNCTIONAL PHENOTYPE

Recent characterization of metabolic immunomodulation has demonstrated the important role metabolism plays in the polarization of macrophages (Reviewed in O'Neill *et al.* [25] and outlined in Fig. 2). Despite recent characterization of macrophage diversity, most current research compares M1 (classically activated macrophages) to M2 (alternately activated macrophages) without the additional complexity of the M2 subgroup functional phenotypes (i.e. M2a, M2b, M2c, M2d). Although basic macrophage homeostasis is maintained in resting and activated (M1/M2) macrophages through persistence of glycolysis metabolism [grey text box, pathway (i), Fig. 2], the M1/M2 activation results in specific energy investment in pathways supportive of the inflammatory function of acute healing (red text boxes, Fig. 2) or supportive of inflammation resolution and tissue repair/regeneration (yellow text boxes, Fig. 2).

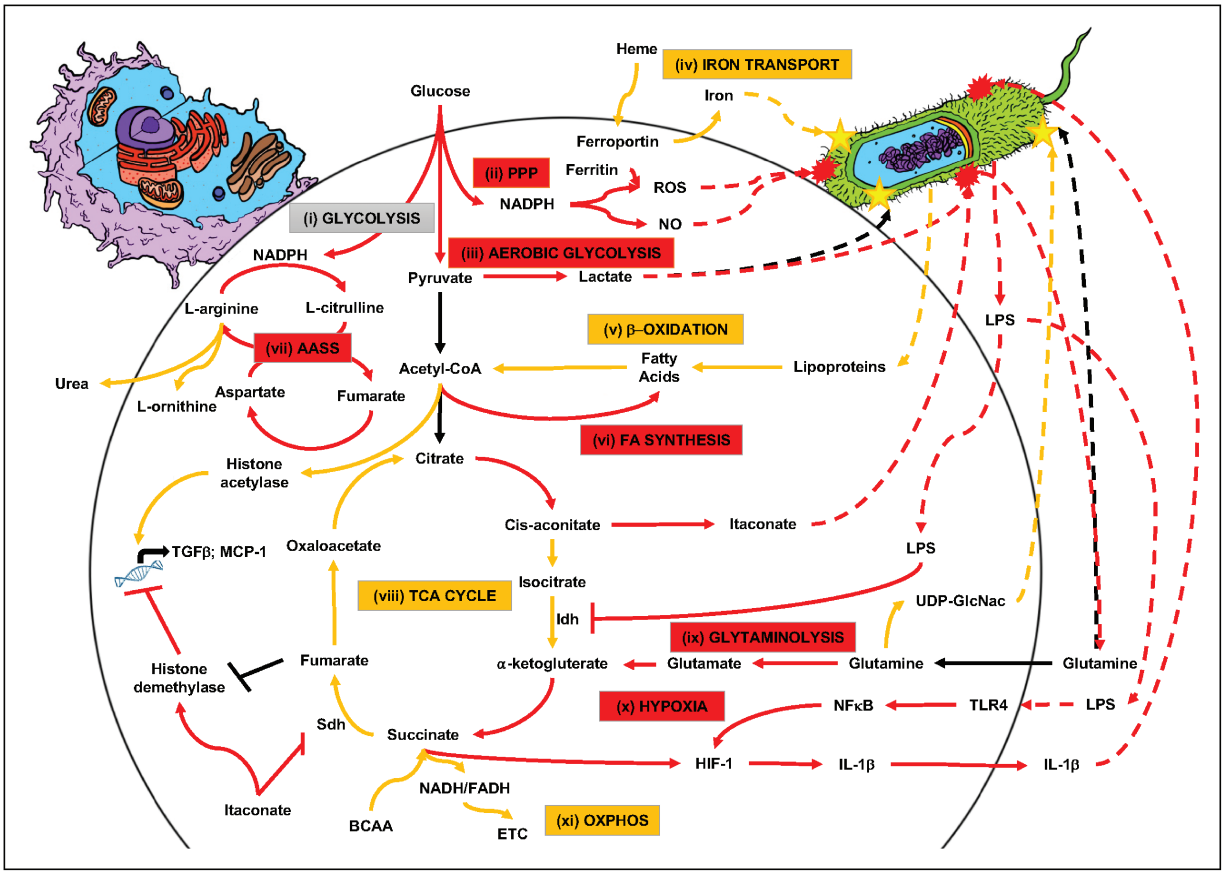

FIGURE 2. Metabolic immunomodulation of macrophage functional phenotype and host–pathogen interactions. Current schematic model of metabolic immunomodulation of macrophage functional phenotype presented as classically activated M1 (red boxes and lines) and alternately activated M2 (yellow boxes and lines), based on currently published research. Grey 'glycolysis' textbox indicative of homeostatic metabolism in M0/M1/M2. Coloured textboxes indicate energetic investment in metabolic pathways during polarization (M1/M2). Solid lines indicate metabolic pathways of interest to polarization (M1/M2; simplified for overall view). Dashed lines indicate pathogen-derived metabolites or pathogen-directed metabolites of host–pathogen metabolic interaction. Yellow stars on pathogen indicate effects of benefit to survival of the pathogen and red explosive shapes on pathogen indicate effects of harm to pathogen survival. Original art by coauthor Tyler Lawton; pathway model based on referenced research in body of review article. BCAA, Branched chain amino acids; ETC, electron transport chain.

As far back as 1958, metabolic pathway contribution to immune function has been described through the Warburg Effect wherein immune activation is coupled to metabolic shift from oxidative phosphorylation (OXPHOS) (pathway xi, Fig. 2) to aerobic glycolysis (pathway (iii), Fig. 2) [26]; however, recent metabolic and transcriptional profiling indicates that this shift to aerobic glycolysis is specific to M1 polarization and oxidative phosphorylation is favoured by M2 polarization [27,28]. A significant metabolic pathway shift during M1 activation to the pentose phosphate pathway (PPP) activates the antimicrobial NADPH oxidase and generates reactive oxygen species (ROS) (pathway (ii), Fig. 2) [29]. The TCA cycle [pathway (viii), Fig. 2] also plays a key role in macrophage polarization, as an intact cycle is associated with the M2 phenotype and an uncoupling of the TCA cycle at both citrate and succinate is characteristic of the M1 phenotype [28,29]. Finally, selective amino acid uptake and metabolism contributes significantly to macrophage polarization. For example, the glutaminolysis pathway [pathway (ix), Fig. 2] and the aspartate-arginosuccinate shunt pathway (AASS) [pathway (vii), Fig. 2] maintain cellular redox homeostasis during ROS and nitric oxide (NO) production, respectively, and is associated with M1 antimicrobial functionality [30,31]. In contrast, arginine flux through the arginase pathway is associated with M2 polarization, immune tolerance and wound healing [32].

Colonizing bacterial interaction with macrophages in the wound also mediates metabolic activation and subsequent M1/M2 polarization [33]. For

example, bacterially derived lipopolysaccharides (LPS) directly inhibit the isocitrate dehydrogenase (Idh), contributing to TCA cycle decoupling, and indirectly lead to macrophage production of the metabolite itaconate, a known antimicrobial metabolite utilized by M1 polarized macrophages [34,35]. In contrast, bacterially derived lipoproteins can feed into β-oxidation metabolism [pathway (v), Fig. 2], providing a key resource for M2 polarization [29,36]. M2 polarization also favours production of uridine diphosphate N-acetyl glucosamine (UDP-GlcNac, Fig. 2) for protein glycosylation of the M2 mannose receptor (CD206) [28], but which can also be co-opted by colonizing bacteria for structural support of the biofilm matrix.

CONCLUSION

Recent observations of macrophage diversity and phenotypic plasticity demonstrate the broad impact of these cells throughout the body [37]; however, temporal progression of wound-associated macrophage function from inflammation to resolution to tissue repair/remodelling indicates this plasticity plays a key role in the normal wound-healing process and dysregulation of this progression contributes to pathogenic nonhealing. Further characterization of macrophage diversity and description of functional phenotype will provide essential leads for novel diagnostic and therapeutic approaches to wound healing [38]. Finally, the recent surge in immunometabolism research has contributed significantly to our understanding of the mechanisms of macrophage polarization [39–41]; however, how the metabolic interactome between wound-associated macrophages and wound-colonizing bacteria contributes to healing and nonhealing remains an area of active discovery.

Acknowledgements

This work was supported with resources and the use of facilities at the Boise VA Medical Center.

The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, the U.S. Department of Veteran Affairs, or the United States Government.

Financial support and sponsorship

C.B.A., T.L. and M.C.B.A. are supported by NIH-NIAID grant R03AI135998 (PI-Ammons) and a subproject to NIH-NIGMS grant P20GM109007 (PD-Stevens, PL-Ammons).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Han G, Ceilley R. Chronic wound healing: a review of current management and treatments. Adv Ther 2017; 34:599–610.
2. James GA, Swogger E, Wolcott R, *et al.* Biofilms in chronic wounds. Wound Repair Regen 2008; 16:37–44.
3. Ayanian JZ, Cleary PD, Weissman JS, Epstein AM. The effect of patients' preferences on racial differences in access to renal transplantation. N Engl J Med 1999; 341:1661–1669.
4. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007; 117:175–184.
5. Huang W, Metlakunta A, Dedousis N, *et al.* Depletion of liver Kupffer cells prevents the development of diet-induced hepatic steatosis and insulin resistance. Diabetes 2010; 59:347–357.
6. Nguyen MT, Favelyukis S, Nguyen AK, *et al.* A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J Biol Chem 2007; 282:35279–35292.
7. Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. J Exp Med 1992; 176:287–292.
8. Sudan B, Wacker MA, Wilson ME, Graff JW. A systematic approach to identify markers of distinctly activated human macrophages. Front Immunol 2015; 6:253.
9. Hesketh M, Sahin KB, West ZE, Murray RZ. Macrophage phenotypes regulate scar formation and chronic wound healing. Int J Mol Sci 2017; 18:pii: E1545.
10. Mosser DM. The many faces of macrophage activation. J Leukoc Biol 2003; 73:209–212.
11. Porta C, Riboldi E, Ippolito A, Sica A. Molecular and epigenetic basis of macrophage polarized activation. Semin Immunol 2015; 27:237–248.
12. ■ Chauhan A, Sun Y, Sukumaran P, *et al.* M1 macrophage polarization is dependent on TRPC1-mediated calcium entry. iScience 2018; 8:85–102.
This original study demonstrates for the first time the role of regulated calcium flux in macrophage polarization to classically activated, pro-inflammatory macrophages.
13. Roszer T. Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. Mediators Inflamm 2015; 2015:816460.
14. ■■ Igarashi Y, Nawaz A, Kado T, *et al.* Partial depletion of CD206-positive M2-like macrophages induces proliferation of beige progenitors and enhances browning after cold stimulation. Sci Rep 2018; 8:14567.
In this original study, the coauthors present molecular interaction between environment of the host, metabolic health and fat deposition, and contribution of alternately activated M2 macrophage functional phenotype in mediating cell–cell interactions.
15. Nishiguchi T, Ito I, Lee JO, *et al.* Macrophage polarization and MRSA infection in burned mice. Immunol Cell Biol 2017; 95:198–206.
16. Eming SA, Werner S, Bugnon P, *et al.* Accelerated wound closure in mice deficient for interleukin-10. Am J Pathol 2007; 170:188–202.
17. Lurier EB, Dalton D, Dampier W, *et al.* Transcriptome analysis of IL-10-stimulated (M2c) macrophages by next-generation sequencing. Immunobiology 2017; 222:847–856.
18. Gabriel VA, McClellan EA, Scheuermann RH. Response of human skin to esthetic scarification. Burns 2014; 40:1338–1344.
19. ■■ Tamura R, Tanaka T, Yamamoto Y, *et al.* Dual role of macrophage in tumor immunity. Immunotherapy 2018; 10:899–909.
Excellent recent review of polarization into functional phenotype of TAMs and the associated interaction of polarized macrophages with the tumour in both beneficial and detrimental pathogenesis.
20. Galdiero MR, Garlanda C, Jaillon S, *et al.* Tumor associated macrophages and neutrophils in tumor progression. J Cell Physiol 2013; 228:1404–1412.
21. ■ Lai YS, Wahyuningtyas R, Aui SP, Chang KT. Autocrine VEGF signalling on M2 macrophages regulates PD-L1 expression for immunomodulation of T cells. J Cell Mol Med 2019; 23:1257–1267.
Original study demonstrating autocrine growth factor signalling in TAMs resulting in suppression of tumorogenic T cells through cell to cell signalling.
22. ■■ Shukla S, Levine C, Sripathi RP, *et al.* The kat in the HAT: the histone acetyl transferase Kat6b (MYST4) is downregulated in murine macrophages in response to LPS. Mediators Inflamm 2018; 2018:7852742.
This original study demostrates the next level of metabolic immunomodulation by connecting metabolic polarization in macrophages through chromatin remodelling and epigenitic regulatory mechanisms.
23. Taylor CT, Colgan SP. Regulation of immunity and inflammation by hypoxia in immunological niches. Nat Rev Immunol 2017; 17:774–785.
24. Wermuth PJ, Jimenez SA. The significance of macrophage polarization subtypes for animal models of tissue fibrosis and human fibrotic diseases. Clin Transl Med 2015; 4:2.

25. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. Nat Rev Immunol 2016; 16:553–565.
26. Warburg O, Gawehn K, Geissler AW. [Metabolism of leukocytes]. Z Naturforsch B 1958; 13B:515–516.
27. Assmann N, Finlay DK. Metabolic regulation of immune responses: therapeutic opportunities. J Clin Invest 2016; 126:2031–2039.
28. Jha AK, Huang SC, Sergushichev A, *et al.* Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. Immunity 2015; 42:419–430.
29. Tannahill GM, Curtis AM, Adamik J, *et al.* Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature 2013; 496:238–242.
30. Murphy C, Newsholme P. Importance of glutamine metabolism in murine macrophages and human monocytes to L-arginine biosynthesis and rates of nitrite or urea production. Clin Sci 1998; 95:397–407.
31. Ghesquiere B, Wong BW, Kuchnio A, Carmeliet P. Metabolism of stromal and immune cells in health and disease. Nature 2014; 511:167–176.
32. Albina JE, Mills CD, Barbul A, *et al.* Arginine metabolism in wounds. Am J Physiol 1988; 254(4 Pt 1):E459–E467.
33. Benoit M, Desnues B, Mege JL. Macrophage polarization in bacterial infections. J Immunol 2008; 181:3733–3739.
34. Meiser J, Kramer L, Sapcariu SC, *et al.* Pro-inflammatory macrophages sustain pyruvate oxidation through pyruvate dehydrogenase for the synthesis of itaconate and to enable cytokine expression. J Biol Chem 2016; 291: 3932–3946.
35. Naujoks J, Tabeling C, Dill BD, *et al.* IFNs modify the proteome of legionella-containing vacuoles and restrict infection via IRG1-derived itaconic acid. PLoS Pathog 2016; 12:e1005408.
36. O'Neill LA, Hardie DG. Metabolism of inflammation limited by AMPK and pseudo-starvation. Nature 2013; 493:346–355.
37. Minutti CM, Knipper JA, Allen JE, Zaiss DM. Tissue-specific contribution of macrophages to wound healing. Semin Cell Dev Biol 2017; 61:3–11.
38. Spiller KL, Koh TJ. Macrophage-based therapeutic strategies in regenerative medicine. Adv Drug Deliv Rev 2017; 122:74–83.
39. Artyomov MN, Sergushichev A, Schilling JD. Integrating immunometabolism and macrophage diversity. Semin Immunol 2016; 28:417–424.
40. Julier Z, Park AJ, Briquez PS, Martino MM. Promoting tissue regeneration by modulating the immune system. Acta Biomater 2017; 53:13–28.
41. Snyder RJ, Lantis J, Kirsner RS, *et al.* Macrophages: a review of their role in wound healing and their therapeutic use. Wound Repair Regen 2016; 24:613–629.