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Novel immunological targets in rheumatic diseases: clues from current therapies

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Many years have elapsed since the discovery of immunomodulators as effective therapeutics for the treatment of rheumatic diseases, and we are still learning about their various mechanisms of action. Here, we provide a concise overview of the most recent discoveries in this field of research, focusing in particular on signaling pathways targeted by therapeutics currently used in the clinic. We highlight areas of investigation that could potentially be explored for the development of new classes of antirheumatic drugs.

Introduction

Now more than ever, immunology is a very exciting and fast-evolving area of research [1]. The availability of sophisticated technologies, together with a wide array of information obtained from comparative biology, has provided scientists with a deeper understanding of the complexity of adaptive and innate immune responses. If we consider just one class of immune cells, the T helper (Th) effector cells, it is easy to see how drastic these changes have been. The 'old' and simplified Th1/Th2 duo representing the yin and yang of the immune response has now been replaced with spider diagrams showing naive T cells in the middle and a number of ramifications leading to Th17, Th22, Th granulocyte macrophage colony-stimulating factor (GM-CSF) and Treg, to name the latest cells reported in the literature [2]. This ever-expanding universe of T cell subsets, as originally termed by Mosmann and Sad [3], has served as inspiration for many scientists who have pushed the boundaries and set themselves the task of discovering similar subsets of B cells [4], dendritic cells [5], macrophages and monocytes [6], to keep the list short.

Thus, it seems that the immune system has become as plastic as the nervous system [7] and, as such, one can barely afford to focus attention on one cell subset without considering the implications that this might have on the others. Not surprisingly, the expansion in so many universes has left scientists (and we the authors of this review) with the very difficult challenge of singling out specific cell targets or signaling pathways as the main focus for drug discovery. Having to face this choice, we decided to focus our attention deliberately on immunological mechanisms that are currently targeted by clinically used therapeutics. Two reasons guided us in this decision: (i) this would be of interest to the readers of *Drug Discovery Today*; (ii) the awareness that the mechanisms of action of long-used and well-known drugs have changed in light of these discoveries. One such example is aspirin: born as a simple inhibitor of prostaglandin production [8], it is now considered a transcriptional regulator [9] as well as a tolerogenic agent for dendritic cells and a modulator of T cell differentiation [10].

Proliferation and survival pathways

The lifespan of immune cells is governed by a wide-range of signaling pathways that are cell-type-specific but that ultimately lead to two main endpoints: apoptosis and/or necrosis. Whereas apoptosis is considered an anti-inflammatory response leading to the resolution of inflammation, necrosis initiates a cascade of reactions that cause tissue damage and malfunction [11].

Several disease-modifying antirheumatic drugs (DMARDs) have been shown to influence immune cell proliferation and apoptosis. Sulfasalazine and its metabolite 5-acetyl salicylic acid have been shown to exert a proapoptotic effect on vascular smooth muscle cells [12] and to accelerate immunocomplex-induced neutrophil apoptosis [13]. Both these effects occurred at concentrations (20–100 mM), considered too low to inhibit nuclear factor (NF)κB activation and found in serum of patients taking a standard oral

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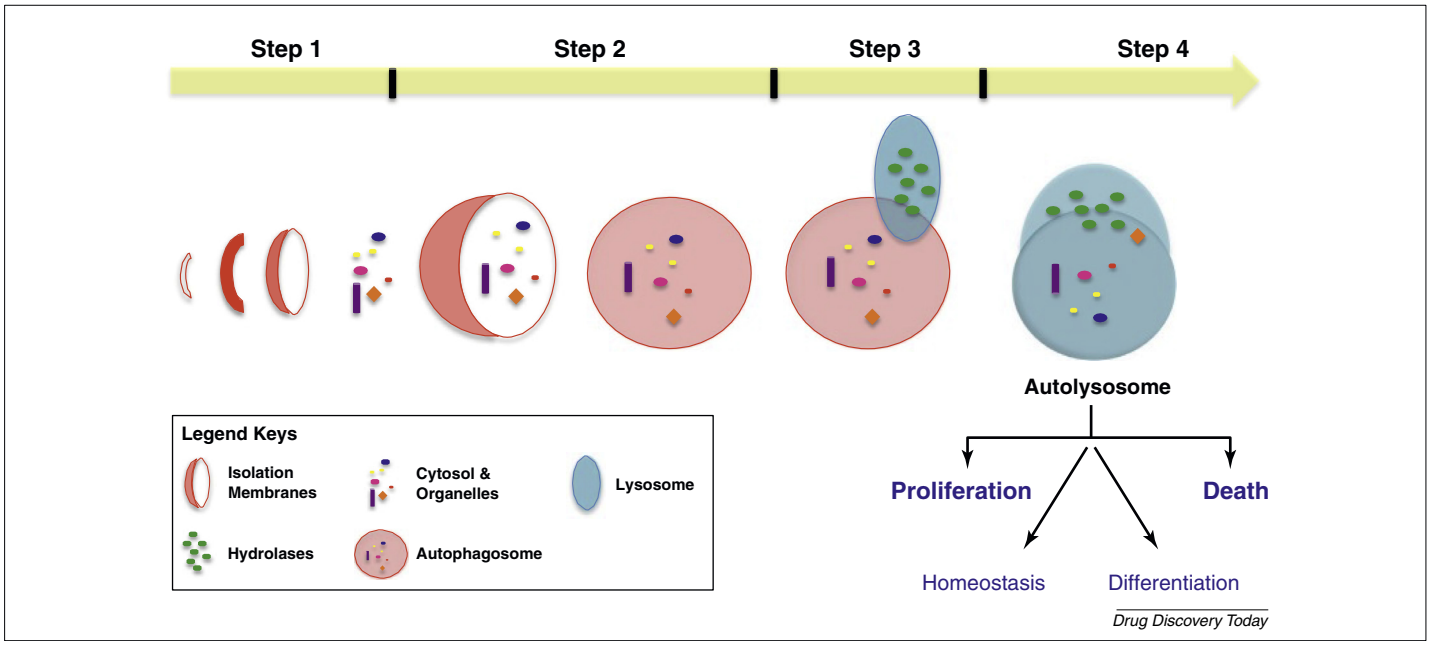

FIGURE 1

Autophagy mechanism. Autophagy, or autophagocytosis, is a catabolic process by which the cell degrades its own components through the lysosomal machinery. It is the major mechanism used by starving cells to create energy. Autophagy plays a part in some cellular processes: proliferation, differentiation, homeostasis and death. A variety of autophagic processes exist but the best known involves the formation of a membrane (called isolation membrane: step 1) that isolates a part of the cytosol including organelles and results in a vesicle (autophagasome: step 2). The autophagasome then fuses to a lysosome (step 3) creating an autolysosome (step 4) in which the degradation of the content occurs.

dose of the drug (3–6 g/day). Because the signaling pathways involved in these effects are not known, it would be interesting to investigate possible interference with the recently described process of autophagy. This is considered as an organic and energy-saving mechanism by which cells degrade and recycle their own components through the lysosomal machinery (Fig. 1) [14].

The translational importance of autophagy in autoimmunity has been supported by several recent studies, reviewed in [15]. Powerful antirheumatic drugs such as glucocorticoids have been shown to induce lymphocyte apoptosis in a receptor-mediated manner [16] and via the expression of a gene encoding a stress response protein called Dig2, RTP801 or REDD1 [17]. Most interestingly, the effect of glucocorticoids on autophagy does not seem to be limited to immune cells. In fact, these drugs have been shown to promote osteocyte autophagy – a mechanism that might explain how steroids alter bone cell fate [18].

Other drugs commonly known to modulate immune cell proliferation include methotrexate (an inhibitor of folic acid metabolism) and leflunomide (an inhibitor of the activity of dihydroorotate dehydrogenase, an enzyme involved in pyrimidine synthesis). Intriguing studies suggest that these compounds might have effects other than regulating immune cell survival. Indeed, *in vitro* studies performed in the presence of an excess of the substrate of the target enzyme have suggested the existence of off-target effects and, hence, alternative mechanisms of action for both molecules.

In the case of leflunomide, tests performed in the presence of uridine have indicated an unexpected inhibition of Janus kinase (JAK)1 and JAK3 activation in T cells [19] or the blockade of cyclooxygenase-2 activity in macrophages and epithelial cells, reviewed in [20]. Similarly, in the case of methotrexate the concomitant administration of folic acid did not diminish the anti-inflammatory properties of this drug, which have been proposed to be caused by the release of adenosine *in vitro* and *in vivo* [21].

Intracelllular and paracellular inflammatory pathways

In a paper published in 1993 by Maini *et al.*, the authors stated: 'In preliminary trials in rheumatoid patients anti-TNF appears to have an impressive effect on indices of disease activity including C-reactive production and serum amyloid-A production. TNF alpha appears to be a relevant therapeutic target in rheumatoid disease' [22].

Twenty years later and anti-tumor necrosis factor (TNF)α drugs are the treatment of choice once synthetic DMARDs start to lose their effects or do not work [23]. Most importantly, anti-TNFα treatment has set the scene for a new class of therapeutics, commonly known as biologics. These drugs are currently seen as the Holy Grail for a wide variety of diseases including rheumatoid arthritis and have paved the way for development of a number of other therapeutics. Indeed, interleukin (IL)-6 and IL-1 receptor antagonists are the next biologics that, as is the case for anti-TNFα, block the effects of these cytokines in a wide variety of pathologies. A great deal of research has been done on the possible mechanisms responsible for the impressive effects of anti-TNFα drugs and include a range of options from reduced localized inflammation and leukocyte recruitment to decreased angiogenesis or selective modulation of T cell subsets such as Th17 and Tregs [24].

In our view, the success behind this story lies within the intrinsic property of the cytokine itself. It is in fact well known that TNFα is one of the first cytokines to be produced and released by immune cells, these being innate cells like macrophages or