feature

Using epidemiology and archaeology to unearth new drug targets for rheumatoid arthritis therapy

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For research scientists in the pharmaceutical industry, rheumatoid arthritis (RA) is considered a target-rich disease. There are multiple cell types contributing to synovial inflammation, including neutrophils, macrophages, mast cells, fibroblasts, T lymphocytes and B lymphocytes. These cells produce a diverse mixture of cytokines, chemokines, proteinases and other inflammatory mediators. Each cell type expresses unique cytokine or chemokine receptors, adhesion molecules, co-stimulatory molecules, pattern recognition receptors and antigen receptors. Clearly, there are enough targets to keep pharmaceutical researchers fully engaged for years to come and scientific advances will continue to add more targets to the list. However, because the cost of drug development is high, not every potential target can be explored in a full drug discovery program. The challenge is to find a logical way to select a small number of targets with the greatest potential for producing efficacious drugs. From analyses of human RA synovium and synovial tissue from animal models of arthritis, much is known about what is involved in the pathology of RA. Unfortunately, we still know very little about why people get RA. A greater understanding of the natural history, epidemiology and genetics of this disease could provide insights for a more logical and directed method of new target selection.

Epidemiology and archaeology

Most textbooks or review articles report a worldwide RA prevalence rate of 0.3–1.5% [1]. In fact, RA prevalence is surprisingly variable among different ethnic or geographical populations [2]. In North America and Europe, the home of most textbook authors, the rate of RA is around 1%, whereas in Africa RA is nearly non-existent, except in areas originally colonized by Europeans. It is also relatively rare in Asia and the Pacific islands (<0.3%). Additionally, the incidence of RA among some Native American populations is unusually high, especially among the Pima Indians (5.3%) and Chippewa Indians (6.8%). These data suggest that a combination of genetic and environmental factors can influence the prevalence of RA.

Although variable among populations, ~1% of people today will suffer from RA. But, has that always been the case? Using a well-defined, stringent set of criteria to differentiate RA from spondyloarthropathy in skeletal remains, Rothschild *et al.* [3] have studied thousands of skeletons from archeological digs throughout the world and have come to the conclusion that RA was nonexistent in Europe before 1785. The archaeological data suggest that for more than 5000 years (6500 to 1000 years ago) RA afflicted

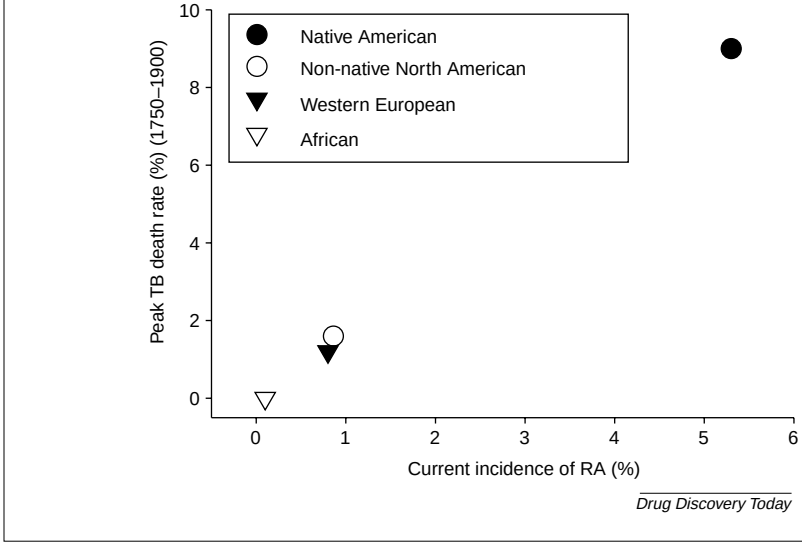

FIGURE 1

Correlation between current RA incidence and TB death rates in the period 1750–1900. Death rates in 1900 for Native Americans caused by TB were obtained from the Fort Qu'Appelle region of Ontario, Canada. RA rates for Native Americans are available for the Pima Indians (5.3%) and the Chippewa Indians (6.8%).

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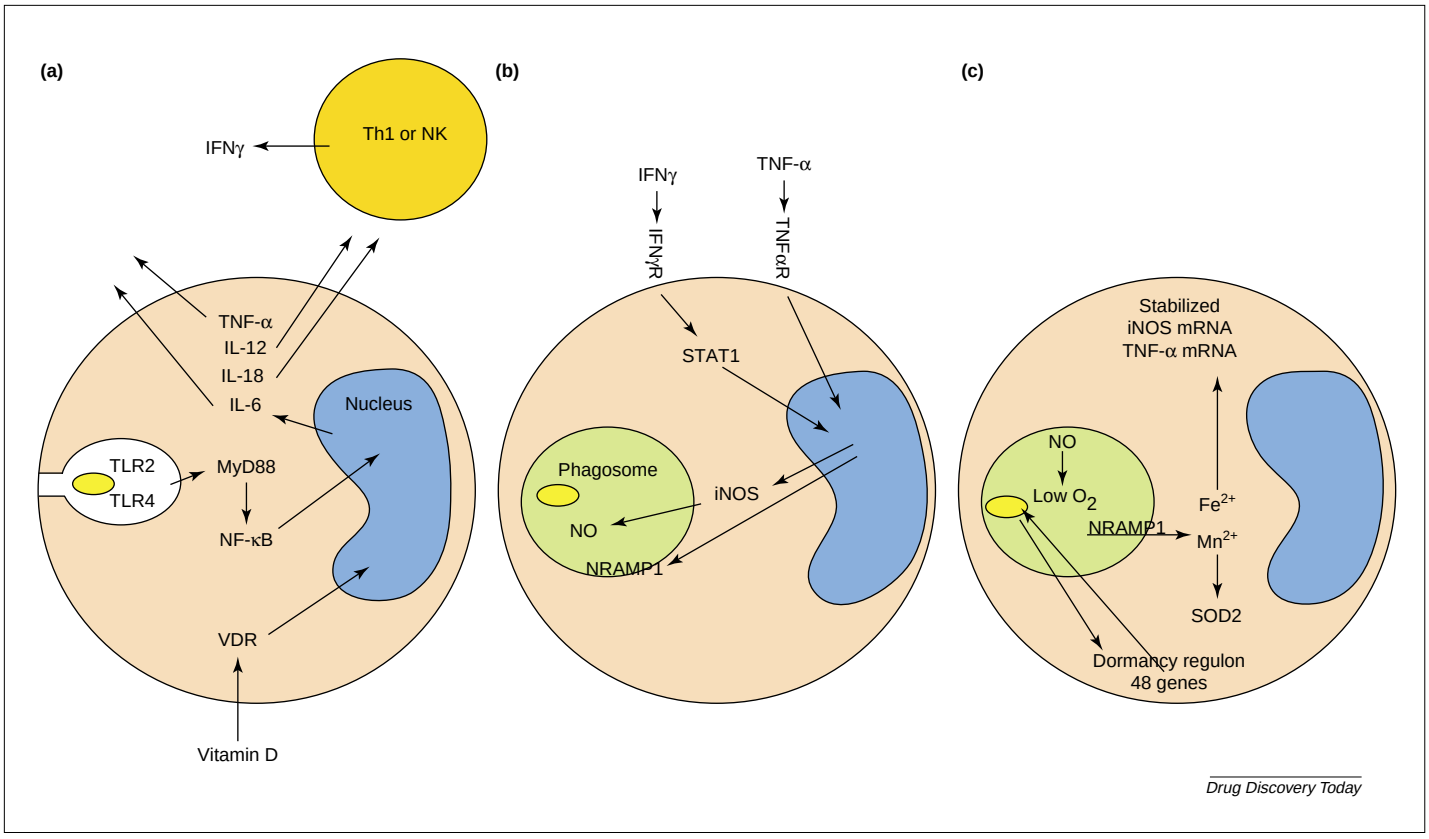

FIGURE 2

Macrophage response to *M. tuberculosis* infection. (a) Macrophage recognition of MTB (yellow) via toll-like receptors initiates a MyD88- and NF-κB-dependent induction of inflammatory cytokine production, resulting in Th1 or NK cell production of IFNγ. **(b)** The synergistic action of IFNγ (via STAT1) and TNF-α on macrophages induces expression of NRAMP1, a divalent cation transporter, and the NO-producing enzyme iNOS. **(c)** NO reduces phagosomal oxygen availability, preventing the MTB from performing aerobic respiration. NRAMP1 transports Fe^{2+} and Mn^{2+} from the phagosome to the cytoplasm. Fe^{2+} stabilizes iNOS and TNF-α mRNA, whereas Mn^{2+} activates SOD2, reducing oxidative damage to the macrophage. Low levels of oxygen and iron induce MTB to initiate transcription of a series of genes that allow it to survive in a dormant state within the phagosome.

only those people living in a small, geographically isolated region of North America [4]. After that time, in a span of less than 1000 years, RA spread to become a worldwide disease afflicting tens of millions of people. This explosive spread from an originally isolated location has suggested that there must be an infectious organism involved.

Tuberculosis connection

Anyone working in arthritis research should already be aware of a connection between RA and *Mycobacterium tuberculosis* (MTB). Two of the workhorse preclinical models of arthritis, collagen-induced arthritis and adjuvant arthritis in rodents, utilize MTB to induce the arthritic response [5]. Moreover, the most promising current therapeutic approach for the treatment of RA, inhibition of tumor necrosis factor alpha (TNF-α), has the unfortunate side effect of increasing the risk of reactive tuberculosis (TB) caused by MTB [6]. The association of RA and TB might be more than coincidence. Although the available data are incomplete, there appears to be a correlation between current rates of RA incidence among various ethnic populations and the death rates caused by TB among those populations 100–200 years ago (Figure 1) [7]. Native Americans suffered the highest recorded TB death rates in 1882 (9000 deaths per 100,000 individuals) [8] and today Native Americans have the highest incidence of RA. Conversely, in 1857 the famous explorer and humanitarian Stanley Livingstone reported that 'tuberculosis did not exist' in Africa [9]. Today, RA incidence rates in Africa are among the lowest in the world. Recorded TB death rates in most European countries and in the USA peaked between 1780 and 1880 at similar levels, between 500 and 2000 deaths per 100,000 individuals [9]. Today, RA incidence rates among these countries are also roughly equivalent.

Infectious diseases are powerful selective forces that can have a substantial impact on human genetics [10]. Random genetic mutations can be 'selected for' or become more frequent within a population because they provide an advantage, helping the carrier of the mutation survive the disease. Sometimes, however, these mutations can also produce phenotypic effects that have nothing to do with the original selective pressure [11–13]. Malaria, for example, a disease caused by the protozoan parasite *Plasmodium falciparum*, has been a leading cause of death for hundreds of years among people living in tropical regions. This constant selective pressure has resulted in the natural selection of several different mutations in the genes encoding hemoglobin [11]. These mutations reduce the ability of *P. falciparum* to infect red blood cells, thereby reducing the incidence of malaria in the carriers of the mutant genes. Unfortunately, this protection does not come without a cost; these same hemoglobin mutations can cause thalassemia or sickle cell anemia in the carriers of the protective genes [11]. Although the cause–effect relationship between malaria and sickle cell anemia is well accepted, this does not mean that *P. falciparum* infection directly causes sickle cell anemia or thalassemia. Similarly, epidemics of cholera hundreds of years ago might have selected for a

mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [12]. Carriers of this mutant gene might have suffered from less-severe diarrhea in response to *Vibrio cholerae* infection than carriers of the normal CFTR gene and, as a result, might have survived cholera epidemics. Again, this does not mean that the *V. cholerae* directly causes cystic fibrosis. As a final example of this phenomenon, it has been hypothesized that the bubonic plague of the middle ages might have selected for the mutation in the chemokine receptor CCR5 that today provides resistance to HIV infection [13]. There is certainly no evidence that the causative agent of bubonic plague, *Yersinia pestis*, has a direct effect on HIV infections.

Just as epidemiological, archaeological and molecular evidence supports links between malaria and sickle cell anemia, cholera and cystic fibrosis, bubonic plague and HIV resistance, similar evidence supports a link between TB and RA [7]. This has led to the hypothesis that epidemics of TB occurring between 100 and 200 years ago might have selected for genetic mutations that increase the risk of developing RA today [7]. Again, it must be stressed that this does not imply that *M. tuberculosis* is a direct cause of RA. On the contrary, if this hypothesis is correct, people with RA should be relatively resistant to TB. Survivors of TB epidemics might have survived because they carried genetic mutations that allowed them to resist infection. Some epidemic survivors would have married and thus passed various combinations of these mutant survival genes to their children. Unfortunately for the survivors' descendents, these genetic mutations can now predispose them to RA. Interestingly, the skeletal remains of the original population of RA-prone Native Americans show no evidence of chronic TB [4]. This is unusual, as skeletal manifestations of MTB infection are fairly common in prehistoric American skeletal remains [14]. However, this observation fits very well with the TB–RA hypothesis: the genetic advantages that protected these ancient people from chronic *M. tuberculosis* infections might have made them uniquely susceptible to RA.

Drug discovery

If RA is a consequence of enhanced resistance to MTB, then understanding the molecular basis of a strong immune response to MTB could help identify good targets for RA therapy. Much has been learned about the immune response to MTB over the past twenty years, by analyzing genetic disorders in humans that make them more susceptible to TB, by employing modern molecular genetics techniques to identify specific genetic polymorphisms among TB-susceptible individuals and by using genetically modified mice to determine whether deleting or overexpressing specific genes will have an impact on MTB resistance or susceptibility [15–18]. MTB infects almost exclusively macrophages and the strength of the immune response made by the infected macrophages dictates whether the infection will be controlled successfully.

Based upon human and murine genetic findings, the following model of innate immunity to MTB has been suggested [15–18] (Figure 2). The initial binding of MTB to macrophages can be mediated by several different receptors including the complement receptors CR1, CR3 and CR4, the mannose receptor, or the type A scavenger receptor [18]. Because multiple receptors can mediate the initial binding event, mutations in any of these receptors does not appear to have a significant impact on rates of TB susceptibility or resistance. Mannose binding lectin (MBL), also known as mannan binding protein or mannose binding protein, is an acute phase protein secreted by the liver that acts as an opsonin, coating bacterial surfaces, activating complement and facilitating phagocytes [19]. Although high MBL levels help protect against most bacterial infections, high MBL levels actually reduce TB resistance. Genetic mutations that result in very low MBL expression occur with high frequency in several populations and are reported to confer resistance to MTB infection [20].

Following MTB attachment to the macrophage cell surface and subsequent phagocytosis, a finely orchestrated immune response begins. Each of the immune mediators in this process, listed in Table 1 and Figure 2, has been found to be crucial for resistance to TB. Mutations in each of these factors have been reported in humans or mice to increase TB susceptibility [15–18]. During phagocytosis, members of the Toll-like receptor (TLR) family of pattern recognition molecules engage MTB cell surface ligands and activate nuclear factor κB (NF-κB) through a myeloid differentiation 88 (MyD88)-dependent pathway. Activation of NF-κB induces the transcription of multiple genes encoding IL-12, IL-18, IL-6, and TNF-α. IL-12 and IL-18 secreted by the TLR-stimulated macrophages induce the production of interferon-γ (IFNγ) from nearby $CD4^+$ T lymphocytes [e.g. T helper 1 (Th1) cells] and natural killer (NK) cells (Figure 2a). The TNF-α and IFNγ act back on the macrophages via TNF receptor (TNFR), IFNγ receptor (IFNγR) and signal transducer and activator of transcription 1 (STAT1) to induce the transcription of additional genes synergistically, including transcription of inducible NO synthase (iNOS) and natural resistance-associated macrophage protein 1 (NRAMP1) (Figure 2b). Expression of iNOS results in the production of nitric oxide (NO), a molecule that is directly toxic to MTB. NO also reduces the availability of O_2 in the phagosome, effectively preventing the bacterium from utilizing aerobic respiration. NRAMP1 transports Mn^{2+} and Fe^{2+} from the phagosome to the cytoplasm. The Fe^{2+} enhances iNOS and TNF-α production, whereas the Mn^{2+} activates superoxide dismutase 2 (SOD2) and reduces oxidative damage to the macrophage. The reduced levels of O_2 and Fe^{2+} within the phagosome will either kill the MTB or induce it to initiate transcription of a 'dormancy regulon', a group of 48 genes that allow the bacteria to become latent and survive for years in such a hostile, low-resource environment (Figure 2c) [21]. The entire response is enhanced greatly if the macrophages have been treated with vitamin D, and vitamin D deficiency or mutations in the vitamin D receptor (VDR) increase susceptibility to MTB infection [16].

Many of inflammatory mediators involved in the immune response to *M. tuberculosis* have also been implicated in RA pathology. Disease association studies have identified mutations, promoter polymorphisms or allelic variations in the genes encoding MBL, VDR, NRAMP1 and IL-18, which are overrepresented in the RA population [20,22–26]. Others, including TLR2, TLR4 and STAT1, are expressed at high levels in human RA synovial tissue [27–29]. Several TB resistance factors, including TNF-α, IL-6, IFNγ, IL-12, iNOS and NF-κB are already the targets of successful RA drug development programs, having produced drugs that are already on the market or in clinical trials. Table 1 lists the factors that have been identified as crucial for resistance to TB infection and also lists any relationship to RA, as a genetic association, synovial tissue expression or successful RA drug target. The overlap of TB resistance factors and RA targets is impressive and suggests that efforts to identify additional TB resistance factors could unearth valuable new targets for RA therapy.

Discussion

Over 50 years ago, J. B. S. Haldane introduced the scientific community to the idea that infectious disease acts as a strong genetic selective force in human evolution [10]. This concept has led to hypotheses linking sickle cell anemia to malaria, cystic fibrosis to cholera, HIV resistance to the bubonic plague and RA to TB. Unfortunately, these types of hypotheses are difficult to test

TABLE 1

TB resistance factors as RA clinical targets

Molecular or cellular target	RA marketed drugs, clinical candidates or preclinical association
Mannose binding lectin	Genetic association [22]
Vitamin D receptor	Genetic association [23]
Toll-like receptors (TLR2, TLR4)	RA tissue expression [27,28]
NF-κB	SPC-839 (Signal-Celgene, preclinical)
TNF-α	Successfully marketed drugs: Enbrel®, Remicade®, Humira®
IL-12 and IL-12R	ABT-874 (CAT/Knoll-Abbott, discontinued); STA-5326 (Synta, preclinical)
IL-18 and IL-18R	Genetic association [26]
IL-6 and IL-6R	Tocilizumab (Chugai-Hoffmann-La Roche, pre-registration)
CD4	IDEC-151 (Biogen-Idec, Phase II)
IFNγ and IFNγR	AGT-1 (Advanced Biotherapy Concepts, Phase II)
STAT1	RA tissue expression [29]
NRAMP1	Genetic association [24,25]
iNOS	GW-274150 (GlaxoSmithKline, Phase II)

and, thus, difficult to disprove. But even an untestable hypothesis can be useful. By considering RA to be a consequence of TB selective pressure, the genetics and the biology of RA begin to make some sense. There might be a 'cause' for RA but it occurred hundreds of years ago. With this point of view established, other chronic inflammatory diseases and autoimmune diseases can be examined with new insight. Perhaps Crohn's disease, multiple sclerosis, diabetes and other diseases are the result of similar selective pressures. Genetic polymorphisms have been identified that are shared by more than one of these diseases, suggesting that there might indeed be an underlying connection [24,25,30,31]. Finding overlapping molecular targets among multiple chronic inflammatory diseases would be a boon to the pharmaceutical industry, because a single drug could be used to treat successfully multiple diseases. By contrast, the increase in TB reactivation among patients treated with TNF-α inhibitors could be a warning worth heeding. The pro-inflammatory mutations in RA and other chronic inflammatory conditions might have arisen as a result of selective pressures by deadly bacteria. For the most part, those bacteria have not gone away.

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