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Historical Overview

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The story of MIF began in 1961 when I came to NYU to work with Lewis Thomas and H. Sherwood Lawrence. Thomas gave me an unpublished manuscript he had received from George and Vaughn describing an *in vitro* method of assessing cellular immunity using capillary tubes. The tubes were filled with guinea pig peritoneal exudates cells and antigens, and then placed in Mackaness-like chambers made of plastic with a shallow chamber sealed at the top and bottom by a round coverslip, and the cells were allowed to migrate onto the bottom coverslip. Cells from animals sensitized to a specific antigen were prevented from migrating by that antigen. This was easy to quantify and thus a great advantage over the inhibition of migration from pieces of lymph nodes that had been previously described many years before by Rich and Lewis.¹ John Vaughn had mentioned to Thomas that he hoped this capillary tube method could be repeated and urged him to try. I found out later that several scientists at NYU had failed in their attempt to repeat it.

Salah Al Askari, a talented urologist from Iraq, and I worked together to try to get this capillary tube method to work. We made a modification simplifying the method by putting only the cells in the tubes and the various antigens in the media filling the chambers, and after a few months had

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it working well. Our early studies were directed to finding out if this migration inhibition assay was related to delayed hypersensitivity. We quickly found that cells were only inhibited when they came from an animal that displayed delayed-type hypersensitivity (DHS) and not if they only produced an antibody.² At this point we urged George and Vaughn to publish their paper, assured it could be repeated.³ We found that, as with the DHS skin test shown by Benacerraf, the migration of macrophages was only inhibited if the DNP protein conjugate was the same as that used to sensitize the animal.⁴ This differed from the reaction of an antibody, which bound to the DNP no matter to what protein it was attached. Later, as Schlossman had shown with the skin test, a DNP-lysine conjugate had to have seven or more lysines to induce and elicit a DHS test or inhibit cell migration while needing only one lysine to react with an antibody.^{5,6}

In trying to understand the mechanism of the migration inhibition, we mixed cells from guinea pigs sensitized with complete Freund's adjuvant (thus sensitive to *M. tuberculosis* antigen or PPD) with non-sensitized cells and found that if as few as 2.5% of the cells came from the sensitized guinea pigs, the migration of the whole population was inhibited by the specific antigen, PPD⁷ (see Fig. 1). As the peritoneal exudates used contained various types of cells, including macrophages, lymphocytes and neutrophils, we wondered what type of cell was the antigen-sensitized cell. It should be recalled that this was just at the time when Gowans was showing the importance of lymphocytes as immunological reactive cells. In subsequent studies, we found that sensitized lymph node lymphocyte preparations, when stimulated by specific antigen, produced a soluble factor that would inhibit the migration of normal peritoneal exudate macrophages.⁸ Independently, and at that time, Bloom and Bennett observed the same phenomenon.⁹ The soluble factor was called MIF for migration inhibitory factor and was the first lymphokine. These studies demonstrated that antigen sensitized lymphocytes produced a soluble factor that mediates a delayed hypersensitivity reaction involving macrophages *in vitro*. Later, the same factor could be produced by stimulating lymphocytes with a mitogen such as concavalin A.¹⁰ It should be stated that MIF did not kill the macrophages; indeed, when observed under high power, one could see that the macrophages were markedly spread out on the glass coverslip, as activated macrophages have been described.

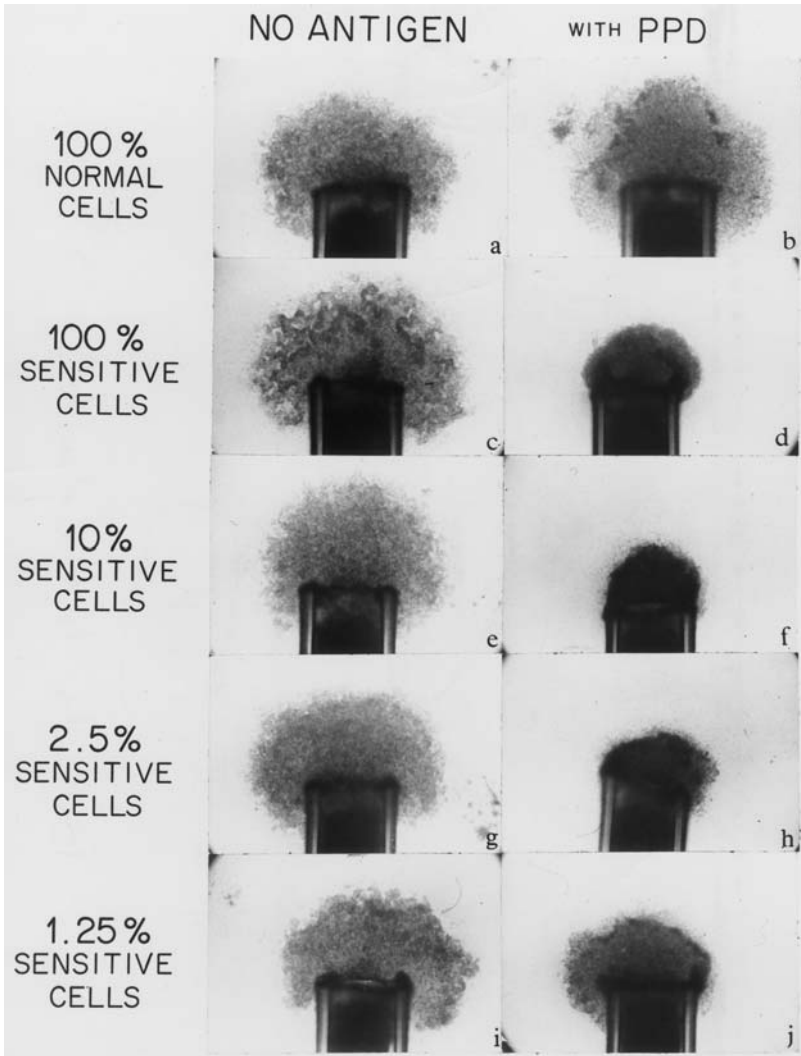


Fig. 1 Capillary tubes filled with guinea pig peritoneal exudates cells. Cells migrate unto coverslip in Mackness-type chamber. On left, no antigen in the media; on the right PPD (tuberculin antigen). Note that when as little 2.5% of the cells in the mixture come from PPD sensitized cells, the whole population is inhibited in migration by the antigen (Ref. 7).

At the time, everyone I talked with thought that MIF had to be an antibody. But it soon turned out that this was not correct. The migration inhibitory property was not removed by anti-antibody columns, and MIF was much smaller than an antibody when eluting from sizing-chromatography columns.¹¹ As more soluble factors with different biologic properties were described, some skeptics postulated that these were different properties of the same factor. In studies using sizing columns or gels, we showed that MIF could be differentiated from chemotactic factors¹² and lymphotoxin.¹³ These mediators were called lymphokines and subsequently cytokines to include similar factors made by cells other than lymphocytes. MIF was also heat stable, thus differing from some complement components, and it was not animal species specific (guinea pig MIF could inhibit mouse or human macrophages), in contrast to interferon-gamma, which is species specific.

MIF was shown to activate macrophages to enhance their glucose metabolism,¹⁴ kill tumor cells, but not normal cells,¹⁵ and kill some bacteria and parasites.^{16,17} It was a macrophage activating factor or a MAF. Peritoneal exudates from animals that had experimental allergic encephalitis were inhibited by brain antigen¹⁸; and the MIF assay showed that patients with glomerulonephritis had cell-mediated immunity (CMI) to glomerular basement membrane antigens,¹⁹ patients with Guillame Barre had CMI to brain antigens²⁰ and patients with drug sensitivity had CMI to the offending drug.²¹ MIF was shown to correlate with DHS in humans.²²

Many years were spent trying to isolate pure MIF without success.

This even once involved trying to get enough lymphocytes by canulating the thoracic duct of a cow housed at the back of the Robert Breck Brigham, a hospital committed to arthritis, to the amazement of the patients who observed the beast from their windows when it was taken out for a daily walk. But we never got enough material. For a review of the studies on MIF prior to its being cloned, see Ref. 23.

A breakthrough came in 1989 when a cDNA encoding a human macrophage migration inhibitory factor (MIF) was isolated, through functional expression cloning in COS-1 cells from a cDNA library prepared from a lectin-stimulated T-cell hybridoma, T-CEMB.²⁴ The 115-amino acid polypeptide encoded by the MIF cDNA (p7-1) was effectively released from the transfected COS-1 cells and yielded readily detectable MIF

activity in the culture supernatant despite the apparent lack of a classical protein secretory sequence. It was different from all proteins in the gene banks at the time. Insertional mutational analysis and elution of MIF activity from polyacrylamide gel slices demonstrated that the 12.5 Kd protein with MIF activity released by the COS-1 cells was encoded by p7-1 (see Fig. 2). The p7-1 cDNA hybridized with a 700-base mRNA expressed by Con-A-stimulated lymphocytes but not unstimulated lymphocytes. It was thought that the availability of the MIF cDNA clone and recombinant MIF would facilitate the analysis of the role of this cytokine in cell-mediated immunity, immunoregulation and inflammation.

The first indication that MIF was not limited to lymphocytes came from a report by Lanahan *et al.* in 1992; they were studying growth factor-induced response genes in mouse 3T3 fibroblasts.²⁵ Some early genes are expressed within minutes after the addition of the growth factor and some,

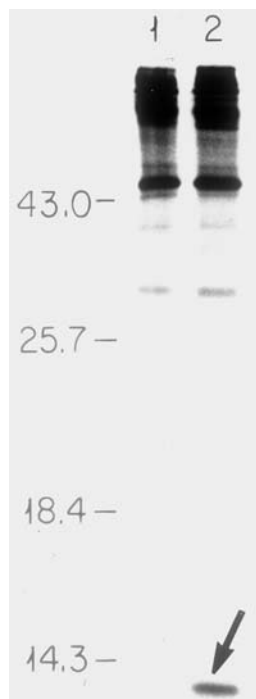


Fig. 2 MIF cloned and separated on a gel in column 2 (Ref. 24). MIF found to be 12.5 K.

called delayed early response genes, are expressed a few hours later. The cDNAs of 13 delayed early response genes were cloned and one, representing 23% of the cDNA, was homologous to human MIF. In fact, it was 100% identical to a mouse MIF we had cloned. Lanahan *et al.* found MIF cDNA in all tissue assayed, including brain, heart, intestine, kidney, liver, lung, ovary seminal vesicles, spleen, testes, thymus and uterus. MIF was also found in differentiating cells of the eye lens.²⁶

Studies from Bucala's group resulted in an avalanche of knowledge about MIF and its role in inflammation and autoimmunity. Bernhagen *et al.* reported in 1993 that pituitary-derived MIF, released by endotoxin, potentiates lethal endotoxemia.²⁷ Further, administration of anti-MIF antibodies neutralized the lethal effect of endotoxin. Subsequently, macrophages were shown to be an important source of MIF.²⁸ LPS and TNF- α increased macrophage release of MIF and MIF in turn increased the release of TNF- α . A fascinating finding was that glucocorticoids, generally known to dampen inflammation, induced MIF release, and that MIF regulated the action of glucocorticoids, decreasing its anti-inflammatory activity.²⁹

The extensive studies on the structure of MIF genes and protein, the role of MIF in infectious diseases, inflammation, autoimmunity, cancer, cell differentiation and atherogenesis, as well as the various mechanisms of MIF action, are covered in detail in this volume. This introduction will briefly summarize some of these.

Structure of MIF. MIF contains 114 amino acids and is 90% homologous in mammalian species so far studied, including human, mouse and cow, and is identical to GIF, a glycosylation inhibiting factor, which has been reported to suppress IgE synthesis.³⁰ Homologues of MIF have been found in fish, chickens, ticks, the nematodes *C. elegans* and *B. malayi*, cyanobacteria and plants.³¹ MIF has been shown to be a homotrimer by crystallography and has considerable structural homology with some microbial enzymes such as oxalocrotonate tautomerase.³² MIF has tautomerase activity, which requires the presence of the amino-terminal proline.³³ It is not known whether some or all of the biologic activity of MIF also requires this amino-terminal proline.

MIF Knockout mice. We produced MIF^{-/-} mice in 1999³⁴ (see Fig. 3). Previous studies showed that mice had only one MIF gene but many pseudogenes.³³ The mouse gene spans less than 0.7kb of chromosomal

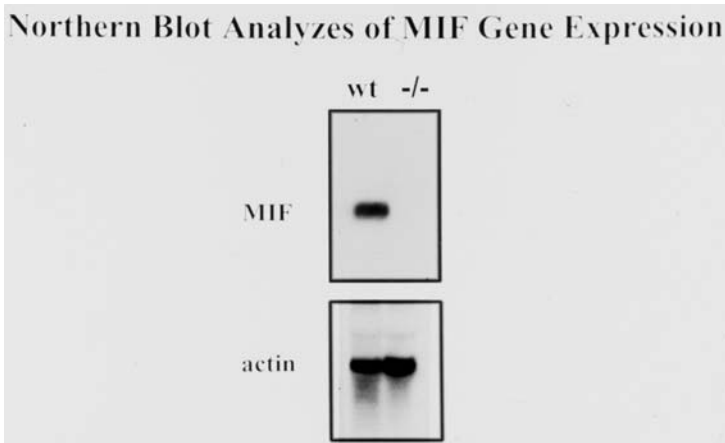


Fig. 3 Northern blot lymphocytes MIF^{-/-} and wild type mice stimulated by concanavalin A. MIF^{-/-} have no MIF mRNA (Ref. 34).

DNA in the middle of chromosome 10 and is composed of three exons. The MIF^{-/-} mouse lacked part of exon 2 and all of exon 3. Analysis of the role of MIF during sepsis showed that the MIF^{-/-} mice were resistant to the lethal effects of high doses of bacteria lipopolysaccharide (LPS; see Fig. 4), or *Staphylococcus aureus* enterotoxin B (SEB) with D-galactosamine and such mice had lower plasma levels of TNF- α than did wild-type mice but normal levels of IL-6 and IL-12.

MIF and infection. To our surprise, MIF^{-/-} mice cleared gram-negative bacteria *Pseudomonas aeruginosa* instilled into the trachea better than wild type mice, and had diminished neutrophil accumulation in their bronchoalveolar fluid compared to wild-type mice.³⁴ Thioglycollate elicited peritoneal exudates in uninfected MIF^{-/-} mice, however, showed normal neutrophil accumulation. MIF knockout mice were shown to clear *E. coli* better than the wild type, as Bozza found that when *E. coli* were injected into the peritoneum of MIF^{-/-} mice, the amount of *E. coli* recovered six hours later was less than one fourth of those found in wild type mice (personal communication; see Fig. 5). Of special interest, Calandra *et al.* found that mice could recover from lethal peritonitis induced by caecal ligation and puncture (CPL) even when the anti-MIF antibody was administered 8 hours after CPL.³⁶ The finding that anti-MIF antibody will prevent the lethal effects of LPS and peritonitis and that MIF^{-/-} mice

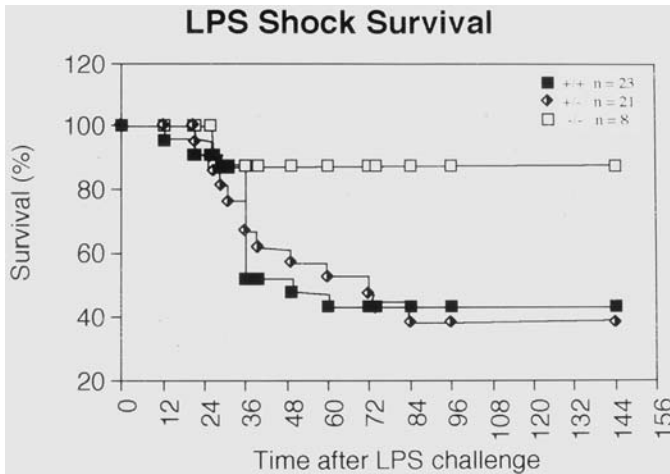


Fig. 4 Survival of MIF^{-/-}, MIF^{+/-} and MIF^{+/+} mice given LPS (Ref. 34). MIF^{-/-}, open squares. This is similar to the survival of mice given anti-MIF in Ref. 27.

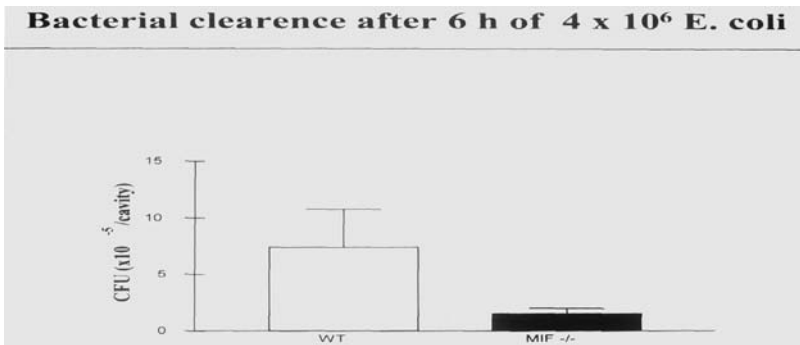


Fig. 5 Number of bacteria in peritoneum 6 hrs after injection 4×10^6 *E. coli*. MIF^{-/-} demonstrate much better clearance (Bozza M., personal communication).

resist the lethal effects of LPS and clear the Gram-negative bacteria *E. coli* and *Pseudomonas. aeruginosa* better than the wild type suggests that neutralizing MIF in such cases of sepsis may be useful. It should be noted that this is not the case for all Gram-negative organisms, as MIF^{-/-} mice were shown to be more susceptible to *Salmonella typhimurium* than wild type mice.³⁷ One explanation for this is that immunity to *Salmonella* requires activated macrophages. As seen below, MIF^{-/-} mice are also

more susceptible to other intracellular infectious organisms such as *Leishmania* and *M. tuberculosis*.

MIF may also play a role in the anemia associated with malaria. *Plasmodium chabaudi*-infected erythrocytes or malarial pigment (hemozoin) was shown to induce the release of MIF from macrophages. MIF was detected in the sera of *P. chabaudi*-infected BALB/c mice, and circulating levels of this cytokine correlated with disease severity. MIF, in concentrations found in patients with malaria, suppressed erythropoietin-dependent erythroid colony formation.³⁸ Further, MIF^{-/-} mice infected with *P. chabaudi* have less severe anemia, improved erythroid development and increased survival compared to wild-type controls.³⁸ Of interest, human mononuclear cells, which had the *MIF* alleles that highly express MIF, produced more MIF when stimulated with hemozoin than cells with *MIF* alleles of low expression; this polymorphism may be partly responsible for determining who gets severe anemia when infected with malaria parasites.³⁸

As described in detail in this volume, MIF^{-/-} mice are more susceptible to *Leishmania major*, *Trypanosoma cruzi* and *Taenia crassiceps*.³⁹⁻⁴¹ For example, compared to wild-type mice, MIF^{-/-} mice infected with *L. major* have larger lesions that persist longer and contain over 100-fold more parasites; their macrophages produce much more IL-6, a cytokine that inhibits the activation of macrophages by interferon-gamma,⁴² and they produce less superoxide and NO (substances important in killing microorganisms), all of which may partly explain the increase susceptibility of these MIF knockout mice.³⁹

In collaboration with Alexandra Sousa and Barry Bloom, we showed that BALB/c MIF^{-/-} (5 generation crossovers) were more susceptible to *Mycobacterium tuberculosis* than wild type mice, the mean survival being 85 days in MIF^{-/-} mice compared to 128 days in wild type controls. ($p < 0.001$) (see Fig. 6).

MIF in autoimmunity and inflammations. Studies, using MIF^{-/-} mice and/or neutralizing anti-MIF antibodies, have shown that MIF plays a proinflammatory role in many inflammatory and autoimmune states. MIF levels have been shown to correlate with fatal outcomes in sepsis.⁴³ Animal models of immune glomerulonephritis,⁴⁴ adjuvant arthritis,⁴⁵ immune type-1-like diabetes,⁴⁶ allergic encephalitis,⁴⁷ acute lung injury or asthma,⁴⁸

Survival of MIF^{-/-} (F5 BALB/c) mice to 10⁵ *M. tuberculosis*

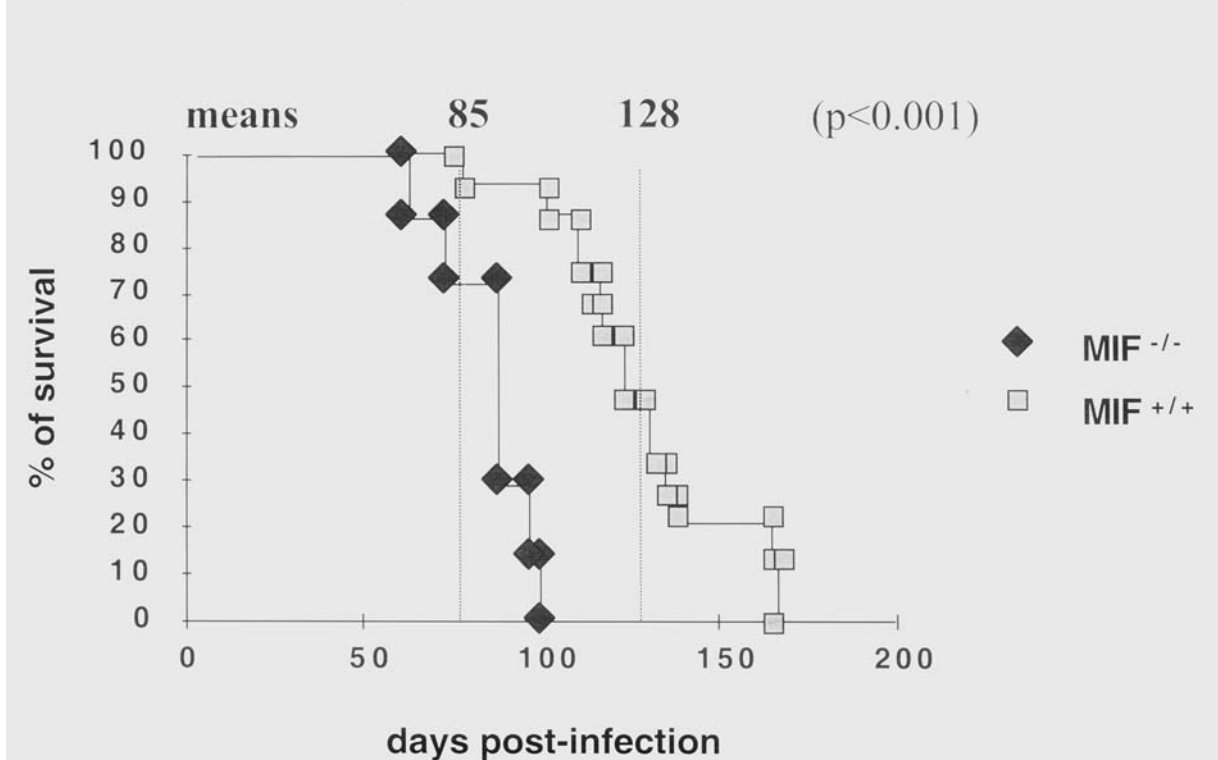


Fig. 6 Survival of MIF^{-/-} and wild type mice after given 10⁵ *M. tuberculosis* (A. Sousa, B. Bloom, JR David, unpublished observations).

allografts,⁴⁹ wound healing⁵⁰ and inflammatory bowel disease⁵¹ have all much milder disease when carried out in MIF^{-/-} animals or when given anti-MIF antibodies (see review, Ref. 31). For example, Rag-2^{-/-} mice, when given CD45^{hi} T cells, develop inflammatory bowel disease, a model for Crohn's disease. However, Rag-2/MIF double knockouts get only minimal disease.⁵¹ Reconstitution of MIF deficient mice with wild-type innate immune cells restored colitis. Further, anti-MIF antibodies could prevent IBD when given at the time of T cell administration and markedly diminish IBD already present when given three weeks after challenge.⁵¹ In contrast, transgenic over-expression of MIF renders mice markedly more susceptible to IBD.⁵² These studies, along with the finding of elevated MIF in patients with Crohn's diseases, suggest that MIF is a new target for intervention in this disease.

MIF and tumor genesis. MIF also plays a role in cancer. It has been shown to increase angiogenesis in the growth of murine lymphomas⁵³ and in sarcoma.⁵⁴ MIF-transfected mice initially had larger tumors than control, but subsequently were shown to cure their tumors. Presumably this was because CTL and NK activity was found to be increased. MIF has been associated with prostatic cancer and been shown to act as a marker for disease progression.^{55,56}

MIF and atherosclerosis. Marked upregulation of MIF was found in vascular endothelial cells of rabbits undergoing atherogenesis in response to a hypercholesterol diet.⁵⁷ Subsequent studies showed that MIF^{-/-} low-density lipoprotein receptor-deficient (LDLr^{-/-}) double knockout mice that consumed an atherogenic diet had much smaller atherosclerotic lesions than similarly fed LDLr^{-/-} mice⁵⁸ (see Fig. 7).

Mechanisms of action of MIF. A number of studies have shed some light on the mechanism of action of MIF. MIF signal transduction is initiated by binding to CD74, a type II transmembrane protein,⁵⁹ and this binding is required for activation of the extracellular signal regulated kinase-mitogen-activated protein kinase cascade (ERK1/ERK2-1/2 MAPK),⁶⁰ for cell proliferation and prostaglandin E2 production. Soluble CD74 will inhibit these MIF mediated reactions.

MIF sustains macrophage proinflammatory function by inhibiting p53.⁶¹ p53 mediates growth arrest and apoptosis, and thus, by suppressing it, MIF may aid in wound healing, but also plays a part in tumor genesis.

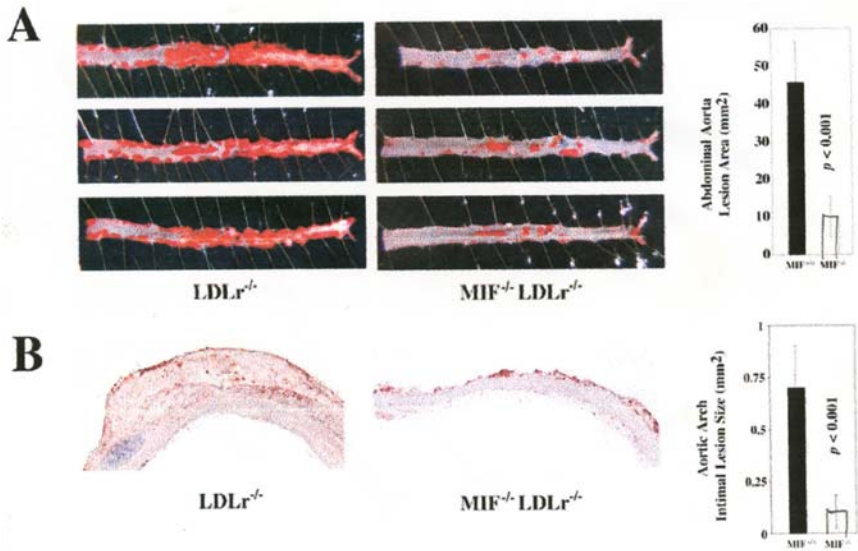


Fig. 7 Much less atheromatous lesions in MIF^{-/-} LDR^{-/-}, than in LDR^{-/-} mice given an atherogenic diet (Ref. 58).

MIF is an essential regulator of macrophage response to LPS and Gram-negative bacteria; it upregulates Toll-like receptor 4 (TLR4) expression, the part of the receptor complex for LPS necessary for signal transduction.⁶² Thus, MIF acts on the transcription factor PU.1, which activates the *Tlr4* gene. LPS acts on the expressed TLR4 on the surface, inducing signal transduction involving NF κ B that subsequently activates the *Tnf- α* gene, causing the macrophage to produce more TNF- α as a response to LPS (see Fig. 8). MIF^{-/-} mice are hyporesponsive to LPS, have less TLR-4 on their cell surface, diminished PU.1 and produce less TNF- α in response to LPS than do wild type controls.

Now to the end of this introduction. Little did we know in the 1960s that MIF, the first lymphocyte mediator/lymphokine/cytokine, or IL-0 if you wish, an *in vitro* measure of delayed hypersensitivity, would have such an impact on so many areas of interest, including inflammation, sepsis and shock, immunity to infectious agents, autoimmunity, wound healing, arterogenesis and cancer. It is now considered an important target for the production of antibodies and anti-MIF drugs and within the next decade,

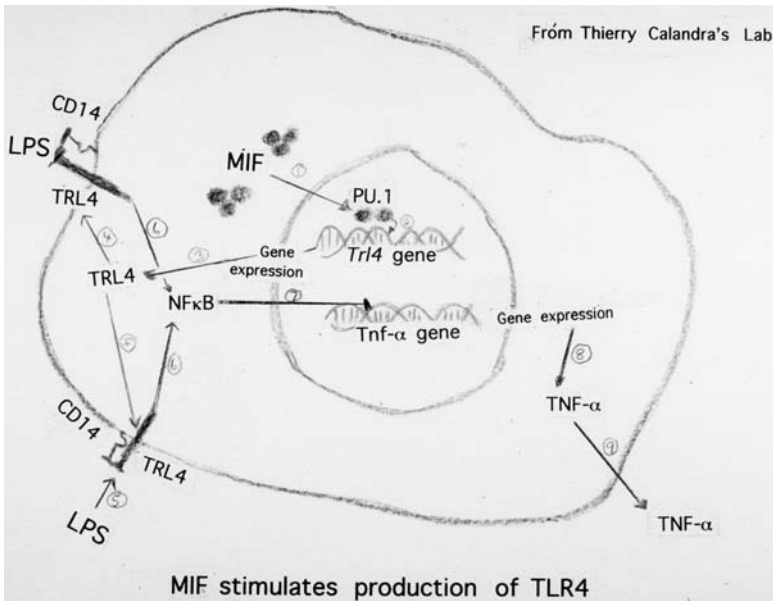


Fig. 8 Diagram of mechanism of MIF stimulating TLR4 (Ref. 62).

we may see if the promise of this Most Interesting Factor, as Richard Bucala likes to call MIF, comes to fruition.

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