Tumor-Derived Macrophage Migration Inhibitory Factor (MIF) Inhibits Immune Reactivity to Neuroblastoma *In Vivo* *

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* The authors have no relationships to disclose.

Background

- Macrophage migration inhibitory factor (MIF), a 12.5 kDa protein, was one of the first cytokines described, more than 40 years ago. Ability to inhibit random macrophage migration.
- MIF is secreted by T cells, macrophages, eosinophils and other tissues including the anterior pituitary gland (in response to stress). Multi-functional protein with several described activities: activation of MAPK signaling, up-regulation of TLR4, promotes expression or pro-inflammatory mediators, counterregulation of glucocorticoids, inhibition of apoptosis, leukocyte recruitment.
- Classically defined as a pro-inflammatory cytokine. However, there are some reports suggesting that MIF can be immune suppressive (reported to inhibit CTL activity and prevent NK lysis). It has been suggested that activity (activation vs. suppression) may be related to protein levels or post-translational modifications of the protein.
- Recently, it has been shown that MIF expression is increased in several malignancies including neuroblastoma, where it appears to function in part as a pro-tumorigenic factor (inactivates p53; sustains ERK1 and ERK2 activation; induces secretion of IL-8 and VEGF).
- To our surprise, we found that mouse tumor-derived MIF was able to strongly inhibit T cell activation *in vitro* (in part, through IFN-γ; Cytokine, 33:188, 2006).

Experimental Hypothesis

Based on our previous results showing that tumor-derived MIF inhibited T cell activation/proliferation *in vitro*, we hypothesized that inhibiting MIF production by tumor cells would increase T cell anti-tumor immunity *in vivo*.

Strategy:

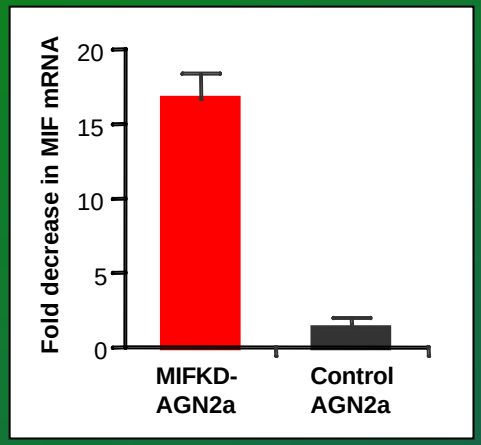
We generated mouse neuroblastoma cells (AGN2a) that had a decreased ability to produce MIF by transducing the cells with short hairpin RNAi lentiviral constructs.

The MIF knockdown (MIFKD) cells were compared to parental and control AGN2a cells with regards to induction of T cell immunity *in vivo*.

MIF Expression in MIFKD-AGN2a Cells

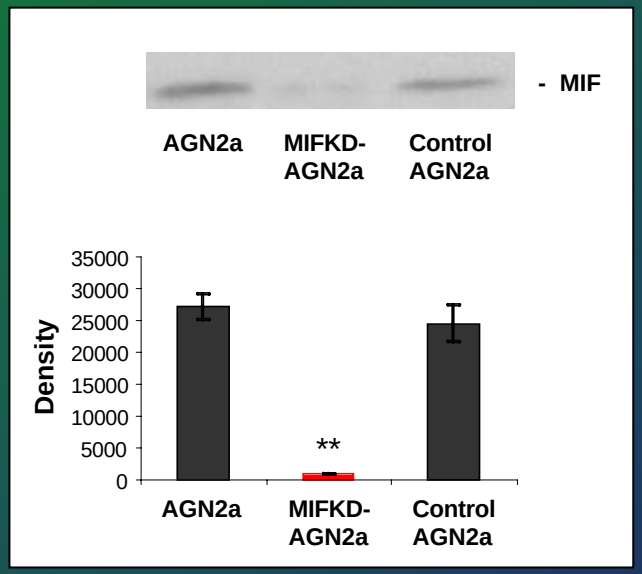
Gene Expression

Real-Time PCR

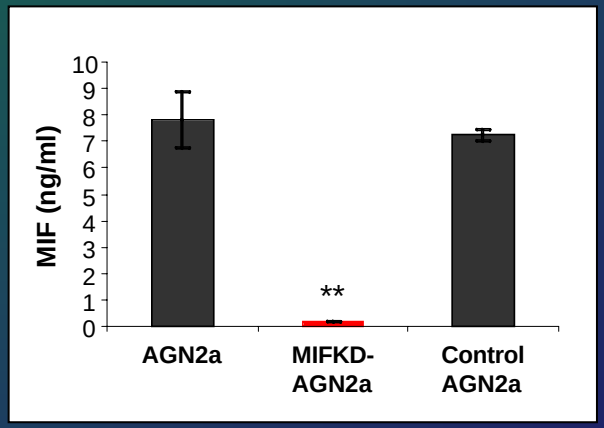


Protein Expression

Western Blot

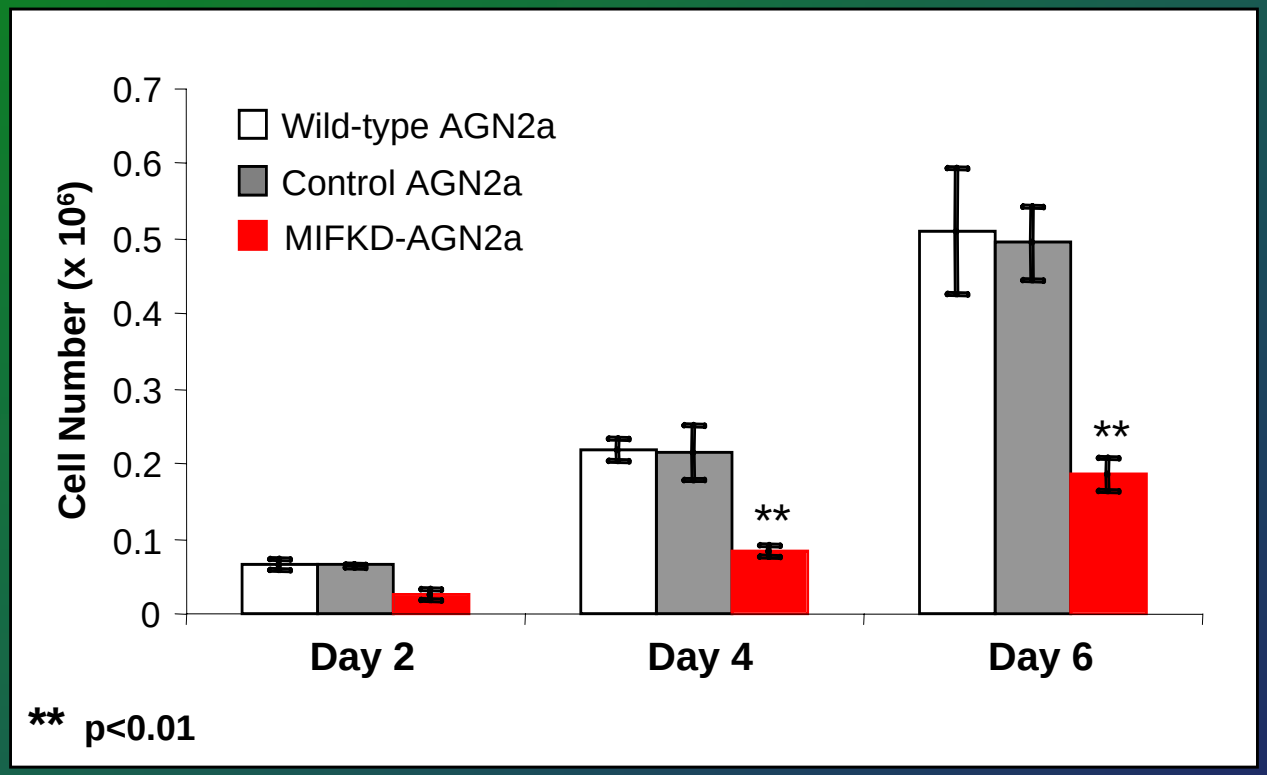


ELISA



** p<0.01

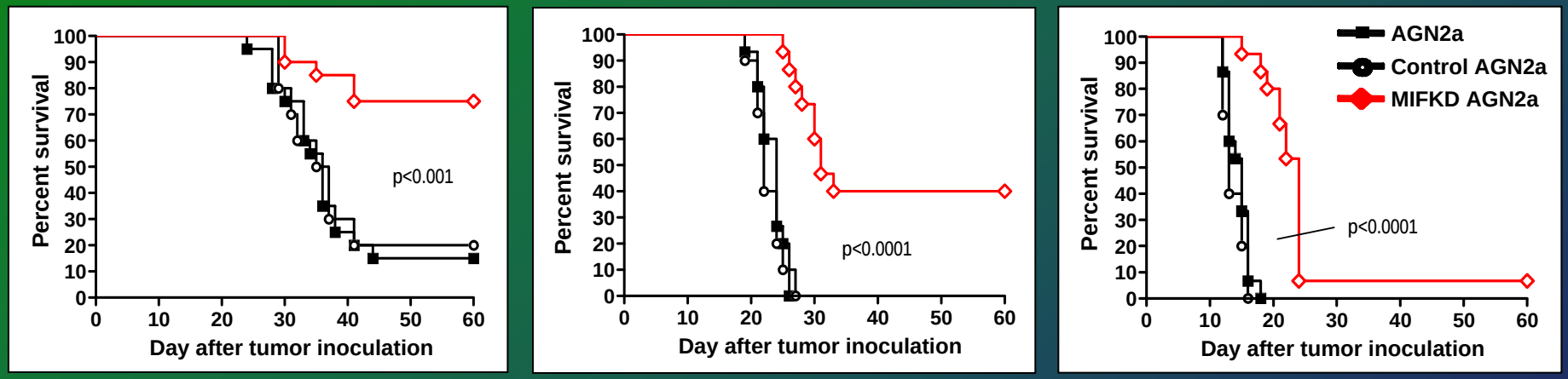
Growth of MIFKD-AGN2a Cells In Vitro



-10^4 cells seeded in culture and cell counts done at the indicated times

Growth of MIFKD-AGN2a Cells In Vivo

10^4 $5x10^4$ 10^6



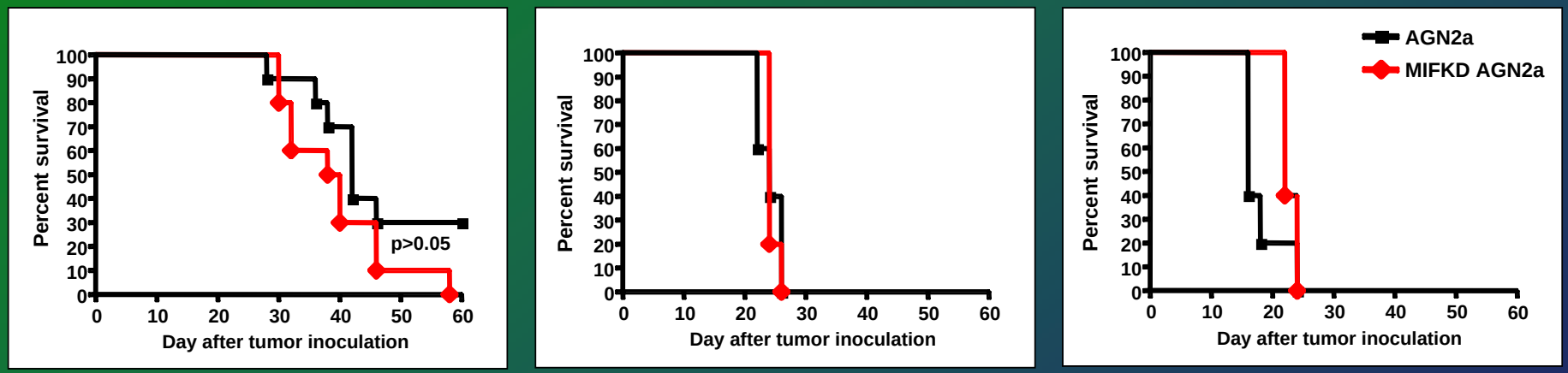
-normal (immune competent) A/J mice were inoculated subcutaneously with the indicated numbers of tumor cells

-mice were considered moribund and euthanized when tumors exceeded 250 mm^2 in size

Increased rejection of the MIFKD tumor cells could be due to decreased growth rate (observed *in vitro*) or due to increased immune reactivity.

Growth of MIFKD-AGN2a Cells in T-depleted Mice

10^4 $5x10^4$ 10^6

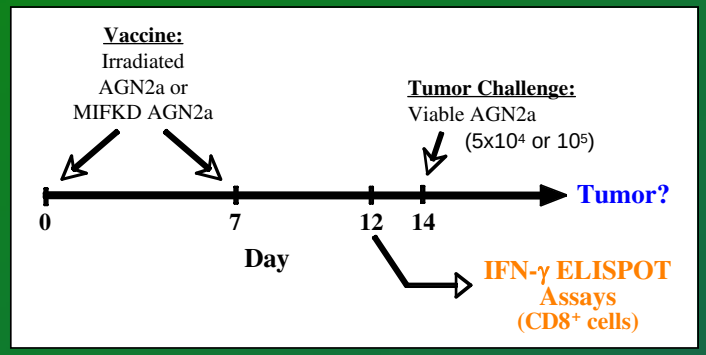


-to deplete T cells *in vivo*, A/J mice were treated i.p. with 500 ug of anti-Thy1.2 mAb two days before tumor inoculation and every four days thereafter until the mice died from tumor progression or until day 60 after inoculation

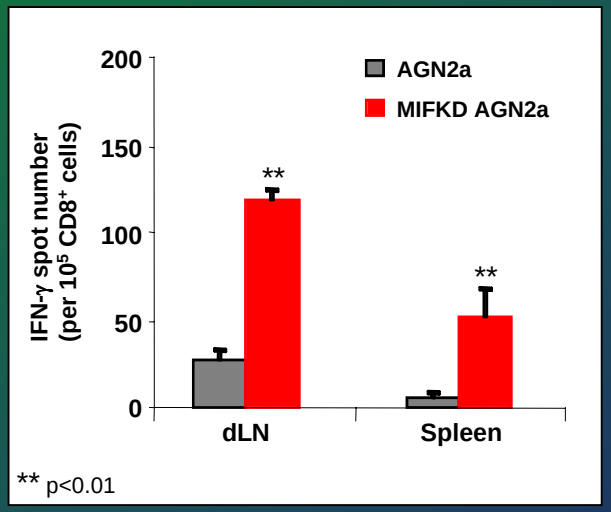
Data indicates that MIF inhibits anti-tumor T cell reactivity *in vivo*.

Can MIFKD-AGN2a Cells Provide Better Anti-Tumor Immunity when Used as a Cell-Based Vaccine?

Experimental Design

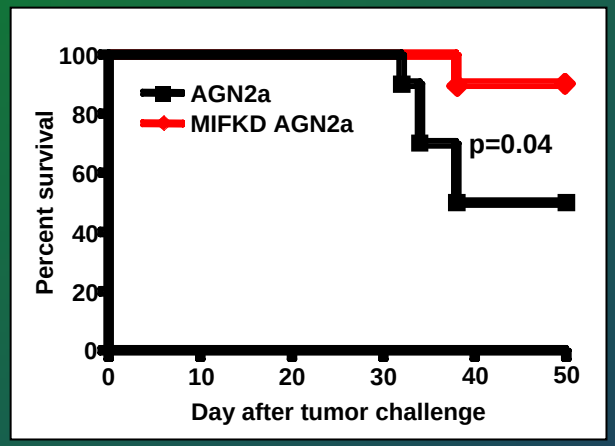


ELISPOT

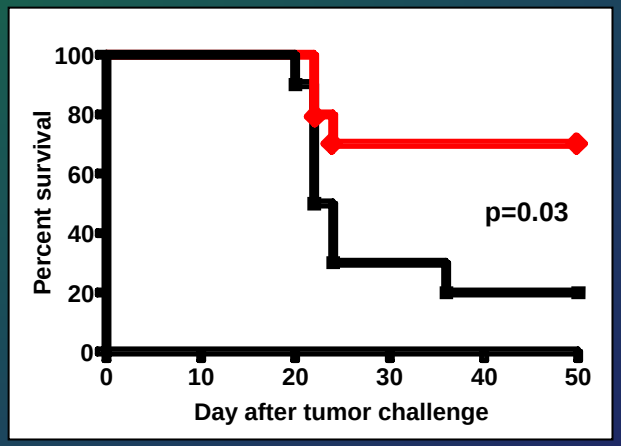


Survival

$5x10^4$



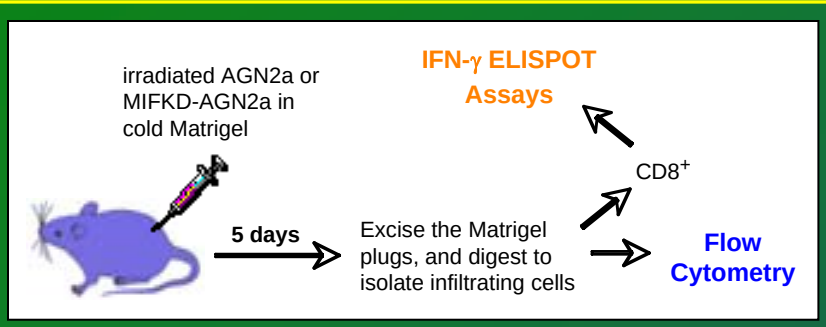
10^5



The MIFKD AGN2a cells were able to serve as a more potent cell-based tumor vaccine.

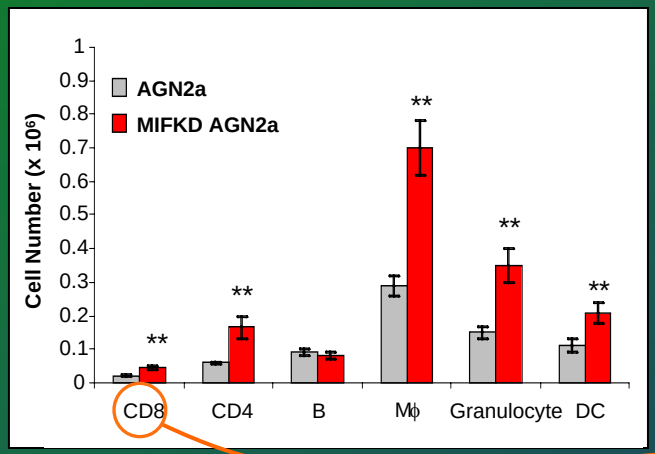
How Do MIFKD-AGN2a Influence Immune Cells at the Site of Vaccination?

Design

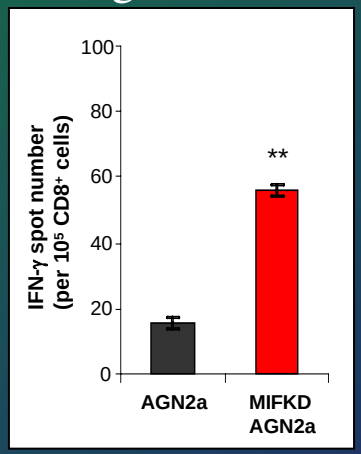


MIF appears to influence the numbers of T cells and other immune cells infiltrating the site of tumor inoculation.

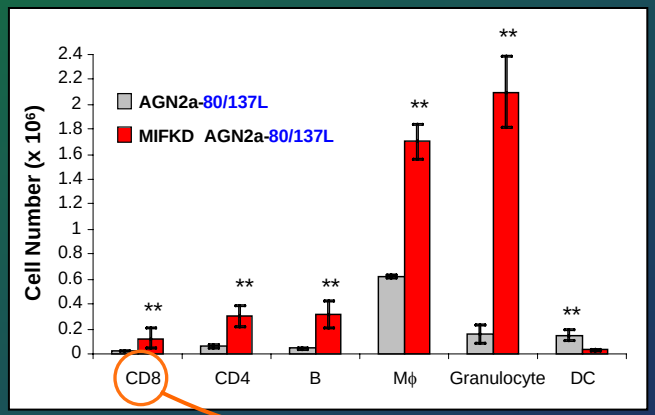
Cell Infiltration

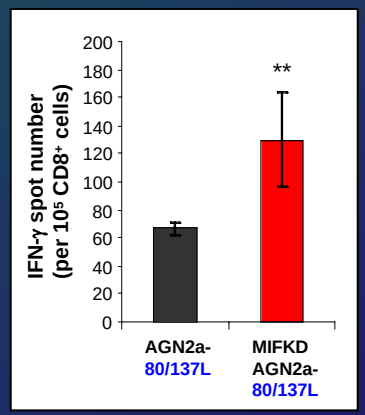


IFN-g ELISPOT



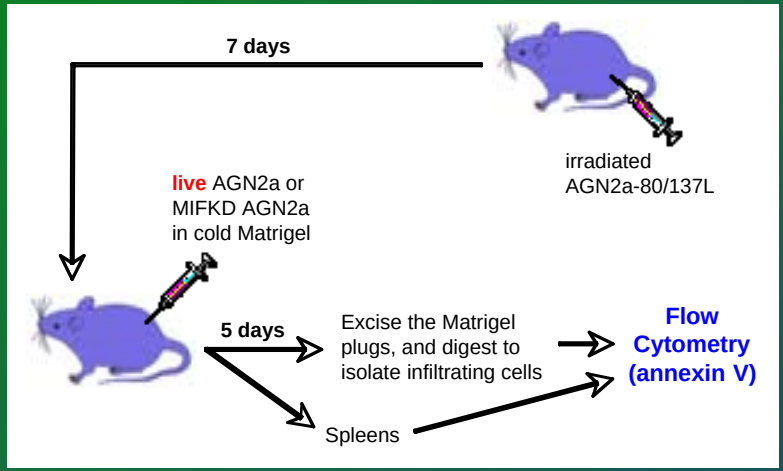
AGN2a cells modified to express CD80 and CD137L

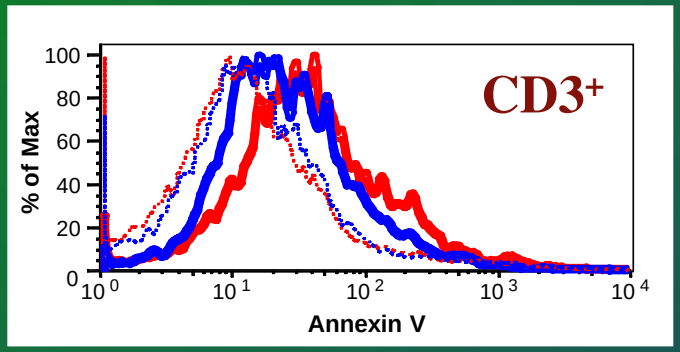


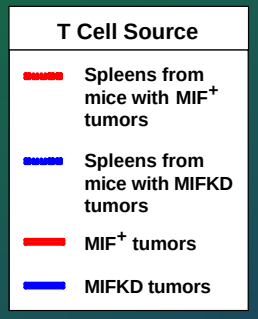


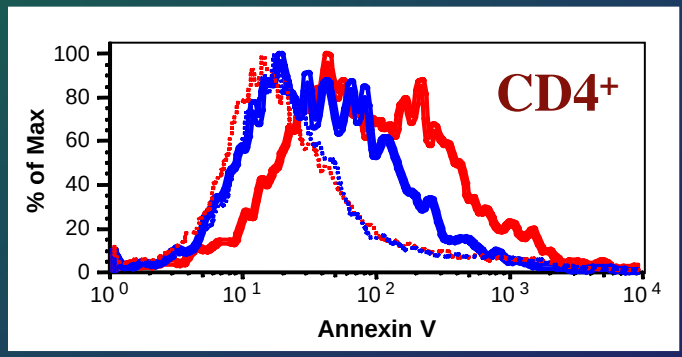
Do Tumor-Infiltrating T Cells Show Signs of Increased Apoptosis due to MIF?

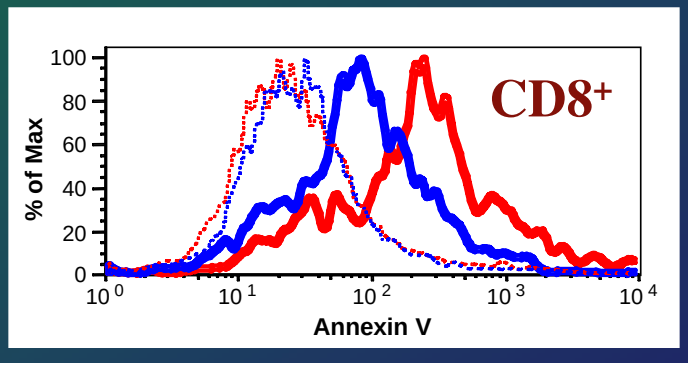
Experimental Design











Tumor-derived MIF may be inhibiting T cells in part by inducing apoptosis.

Conclusions

- While tumor-derived MIF may function, in part, as an autocrine growth factor for mouse neuroblastoma, our results indicate that tumor-derived MIF also inhibits anti-tumor T cell reactivity *in vivo*.

- When mouse neuroblastoma cells were administered as a vaccine, tumor-derived MIF inhibited the accumulation of tumor-reactive T cells and several other potential immune effector cells at the site of vaccination.

- Tumor-derived MIF may inhibit anti-tumor T cell immunity *in vivo* by inducing apoptotic cell death. Our previous *in vitro* data suggests that this occurs in part through an IFN-γ pathway, but the specific mechanism(s) need to be investigated further.

- Summary: These results suggest that the MIF produced by this murine neuroblastoma contributes to immune evasion.

Acknowledgements

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Molecular Signalling Pathways Impacted by MIF

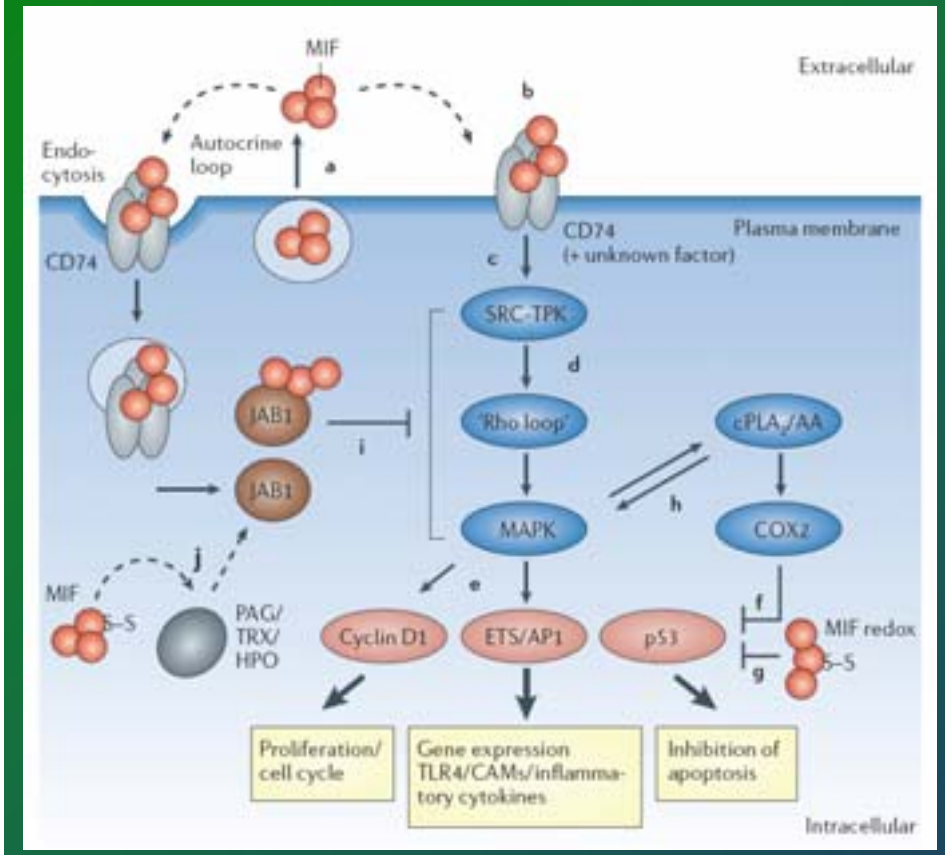

Figure 2 | **Molecular mode of action of MIF.** Macrophage migration inhibitory factor (MIF) regulates cell activation through extracellular, receptor-mediated signalling pathways, and intracellular interactions. Extracellular MIF, which includes MIF derived from intracellular stores in an autocrine fashion (**a**) interacts with cell surface CD74 (**b**). MIF activates the extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (MAPK) pathways in a CD74-dependent manner (**b**,**c**). Intermediate protein regulators between the MIF–CD74 interaction and subsequent intracellular events have yet to be characterized (**c**). Downstream events include a SRC-family-type tyrosine kinase and RhoGTPase–Rho kinase–myosin light chain kinase (MLCK)–integrin–focal adhesion kinase (FAK) activation loop (**d**). This results in the activation of cyclin D1, ETS domain-containing transcription factors (ETS) and activator protein 1 (AP1) transcription factors, leading to downstream effects on cell cycle and gene expression including cell-adhesion molecules (CAM) and Toll-like receptor 4 (TLR4) (**e**). p53-dependent inhibition of apoptosis by MIF can also be initiated through the MIF–CD74 interaction, which involves the downstream activation of cytosolic phospholipase A_2 ($cPLA_2$), generation of arachidonic acid (AA), and activation of cyclooxygenase 2 (COX2) (**f**). Apoptosis induced under conditions of pro-oxidative stress is further inhibited by MIF's antioxidant activity, which depends on intramolecular disulphide (S-S) bonds (**g**). Arachidonic acid can in turn lead to MAPK activation and AP1-regulated gene expression (**h**). Certain intracellular proteins directly interact with MIF. High concentrations of endocytosed MIF bind to c-Jun activation domain binding protein 1 (JAB1) and negatively regulate MIF signalling through MAPKs (**i**). Intracellular MIF also possibly regulates JAB1 and other cell functions through enzymatic regulation via peroxiredoxin 1 (PAG), thioredoxin (TRX) or hepatopoietin (HPO) (**j**). In addition, MIF can bind to MLCK, and constitutive photomorphogenesis 9 signalosome subunit 6.

From: Morand et al., Nat. Rev. Drug Discovery, *5*:399, 2006