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(54) Title: TNF SIGNALING IN MYXOMA VIRUS TREATMENTS

(57) Abstract: Cells that are deficient in TNF responsiveness are susceptible to infection with a myxoma virus. People infected with myxoma virus infection are treated using an activator of tumor necrosis factor receptor. MT-2 deficient myxoma viruses are useful in treating cancer.



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TNF SIGNALING IN MYXOMA VIRUS TREATMENTS

BACKGROUND OF THE INVENTION

Myxoma virus (MV) is a rabbit-specific poxvirus. In other words, MV only infects rabbits in natural settings. Previous studies have shown that the species barrier for MV in mice is maintained by interferon (IFN) signaling. While MV is capable of infecting humans, MV cannot cause disease in humans even if it is injected on purpose. However, the molecular mechanism underlying the species restriction in humans was not known.

TNF has been shown to have direct antiviral activity against some DNA and RNA viruses (Mestan, et al., Nature (October 1986) 323:816-819; Wong and Goeddel, Nature (October 1986) 323:819-822), but the antiviral activity of TNF against myxoma in human cells was not previously known.

DESCRIPTION OF THE INVENTION

This invention is based on the identification of the TNF α -NF κ B signaling cascade as the major component for the myxoma virus (MV) species barrier in humans. The species barrier for myxoma virus in humans is TNF α -NF κ B dependent.

This invention provides a method for identifying cells susceptible to infection with a myxoma virus, comprising assaying a test cell for responsiveness to tumor necrosis factor (TNF), wherein a cell that is deficient in TNF responsiveness is identified as likely being susceptible to myxoma virus infection. In accordance with the method of this invention, any kind of cell can be tested. In one embodiment, the cell is a human cell, for example a human cancer cell. Preferably the TNF is TNF-alpha.

This invention provides a method of treating a human subject having a myxoma virus infection, comprising administering an activator of tumor necrosis factor receptor to

the subject in an amount effective to treat the subject. Preferably the TNF receptor activator is TNF, more preferably TNF-alpha. Other activators can also be used, such as agonist antibodies and synthetic peptides. Activation of the TNF receptor leads to nuclear translocation of the transcription factor NF-kB. Thus other agents capable of causing the nuclear translocation of NF-kB can be used in a manner analogous to the use of TNF. These agents include, but are not limited to, Fas ligand, and agonists of Toll-like receptors (TLRs). The particular TLRs and their agonists that lead to the nuclear translocation of NF-kB are well known within the art.

This invention provides a method of treating a human subject having cancer, comprising administering to the subject a MT-2 deficient myxoma virus in an amount effective to treat the subject. Myxoma and other viruses have evolved mechanisms to antagonize TNF signaling in a species-specific (natural host) manner, leading in part to the manifestation of the natural tropism. This antagonism is not required in cells that are, in and of themselves, defective in TNF response, such as human tumor cells, and therefore the virus tropism is extended to these types of cells. One example of this antagonism is the myxoma M-T2 viral protein, which can bind to and sequester TNF ligand. Because tumor cells can become TNF-resistant, these viruses may grow and kill neoplastic cells from a non-natural host, the M-T2 viral protein is dispensable for growth in human tumor cells.

This invention provides a method of treating a human subject having cancer, comprising administering to the subject a myxoma virus that expresses tumor necrosis factor in cells of the subject. In an embodiment of this method, the expressed tumor necrosis factor is human tumor necrosis factor, preferably tumor necrosis factor-alpha. In an embodiment of this method the myxoma virus is MT-2 deficient.

DESCRIPTION OF THE FIGURES

Figure 1. Endogenous activated Akt levels in human glioma cells

Figure 2. Viral replication efficiency of the various vMyx-hrKOs and controls in human glioma cell lines.

Figure 3. Secreted early and late viral gene expression indicates that some of the vMyx-hrKO are unable to transit from early to late gene expression.

Figure 4. Selected single step growth curves.

Figure 5. Cell-based cytotoxicity assay

Figures 6-30.

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CLAIMS

What is claimed is:

1. A method for identifying cells susceptible to infection with a myxoma virus, comprising assaying a test cell for responsiveness to tumor necrosis factor, wherein a cell that is deficient in tumor necrosis factor responsiveness is identified as likely being susceptible to myxoma virus infection.
2. The method of claim 1, wherein the cell is a human cell.
3. The method of claim 2, wherein the cell is a human cancer cell.
4. The method of claim 1, wherein the tumor necrosis factor is tumor necrosis factor-alpha.
5. A method of treating a human subject having a myxoma virus infection, comprising administering an activator of the tumor necrosis factor receptor to the subject in an amount effective to treat the subject.
6. The method of claim 5, wherein the activator of tumor necrosis factor receptor is tumor necrosis factor.
7. The method of claim 6, wherein the tumor necrosis factor is tumor necrosis factor-alpha.
8. A method of treating a human subject having cancer, comprising administering to the subject a MT-2 deficient myxoma virus in an amount effective to treat the subject.
9. A method of treating a human subject having cancer, comprising administering to the subject a myxoma virus that expresses tumor necrosis factor in cells of the subject.

10. The method of claim 9, wherein the expressed tumor necrosis factor is human tumor necrosis factor.
11. The method of claim 10, wherein the expressed human tumor necrosis factor is tumor necrosis factor-alpha.
12. The method of claim 9, wherein the myxoma virus is MT-2 deficient.

FIGURE 1

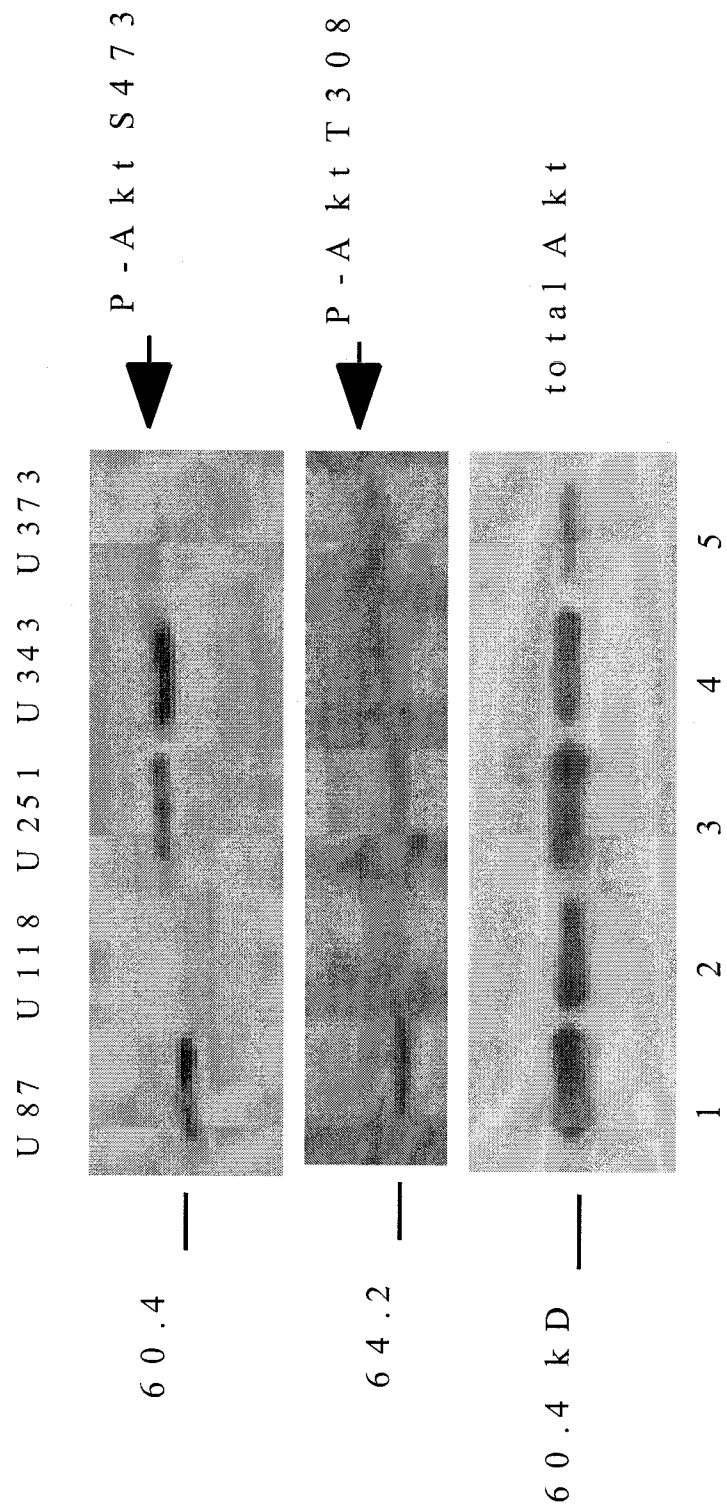


FIGURE 2

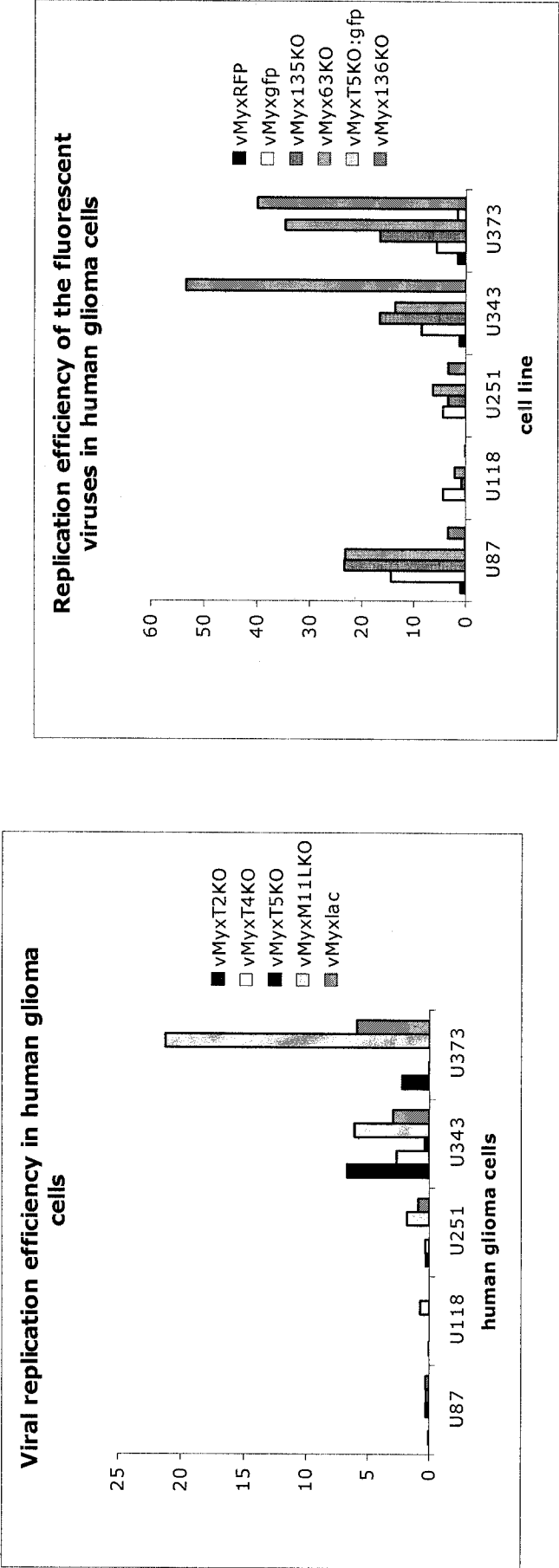


FIGURE 3

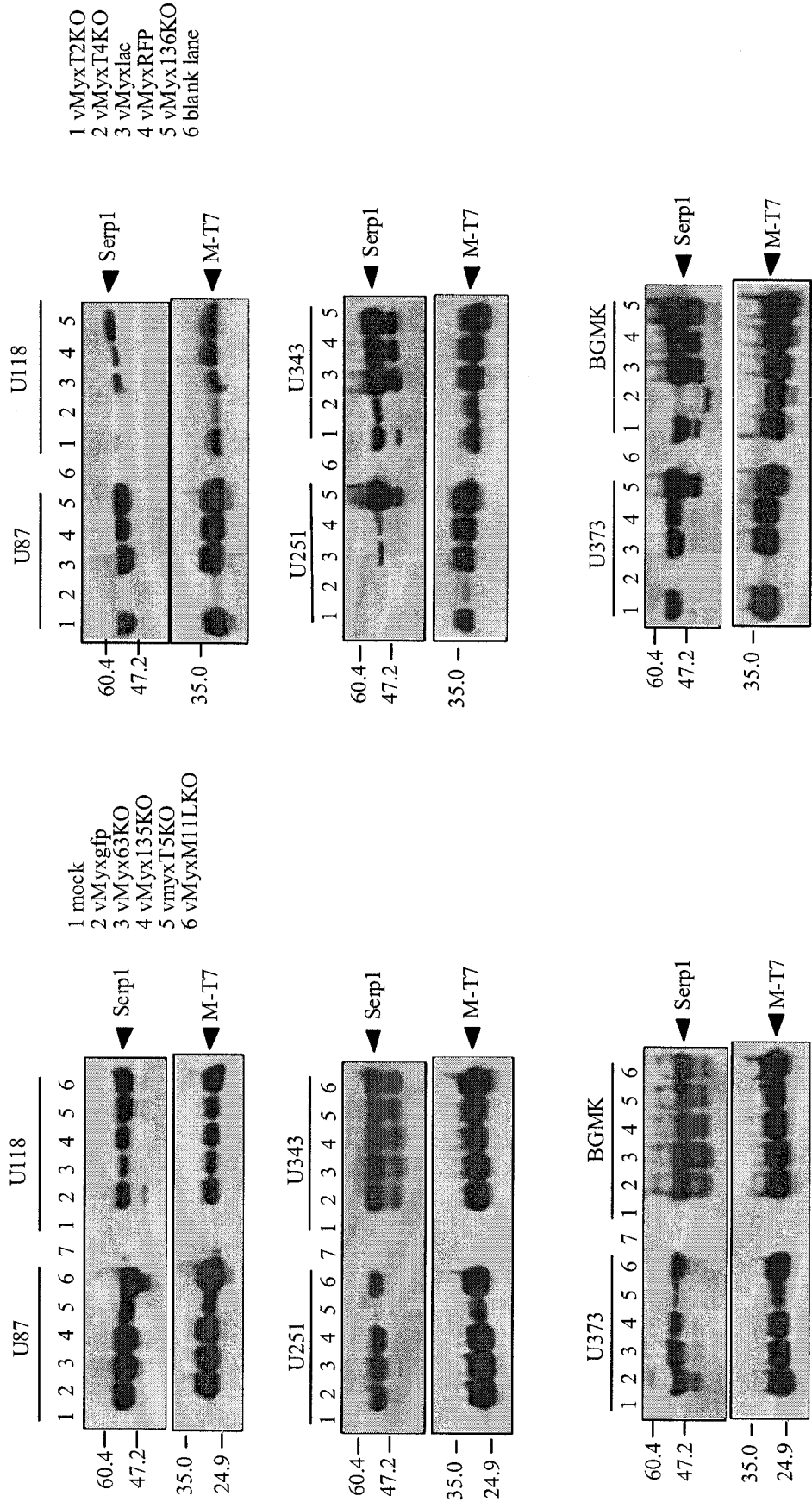


FIGURE 4

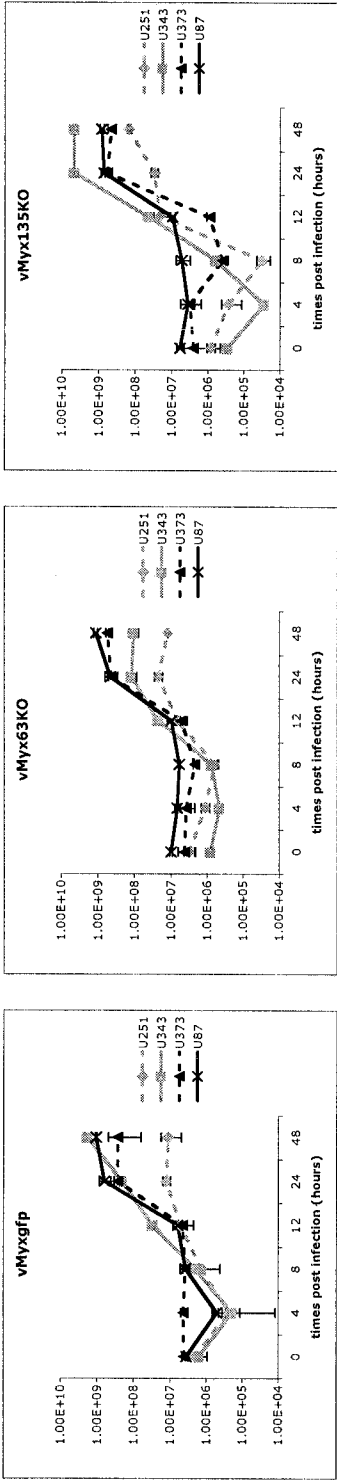


FIGURE 5

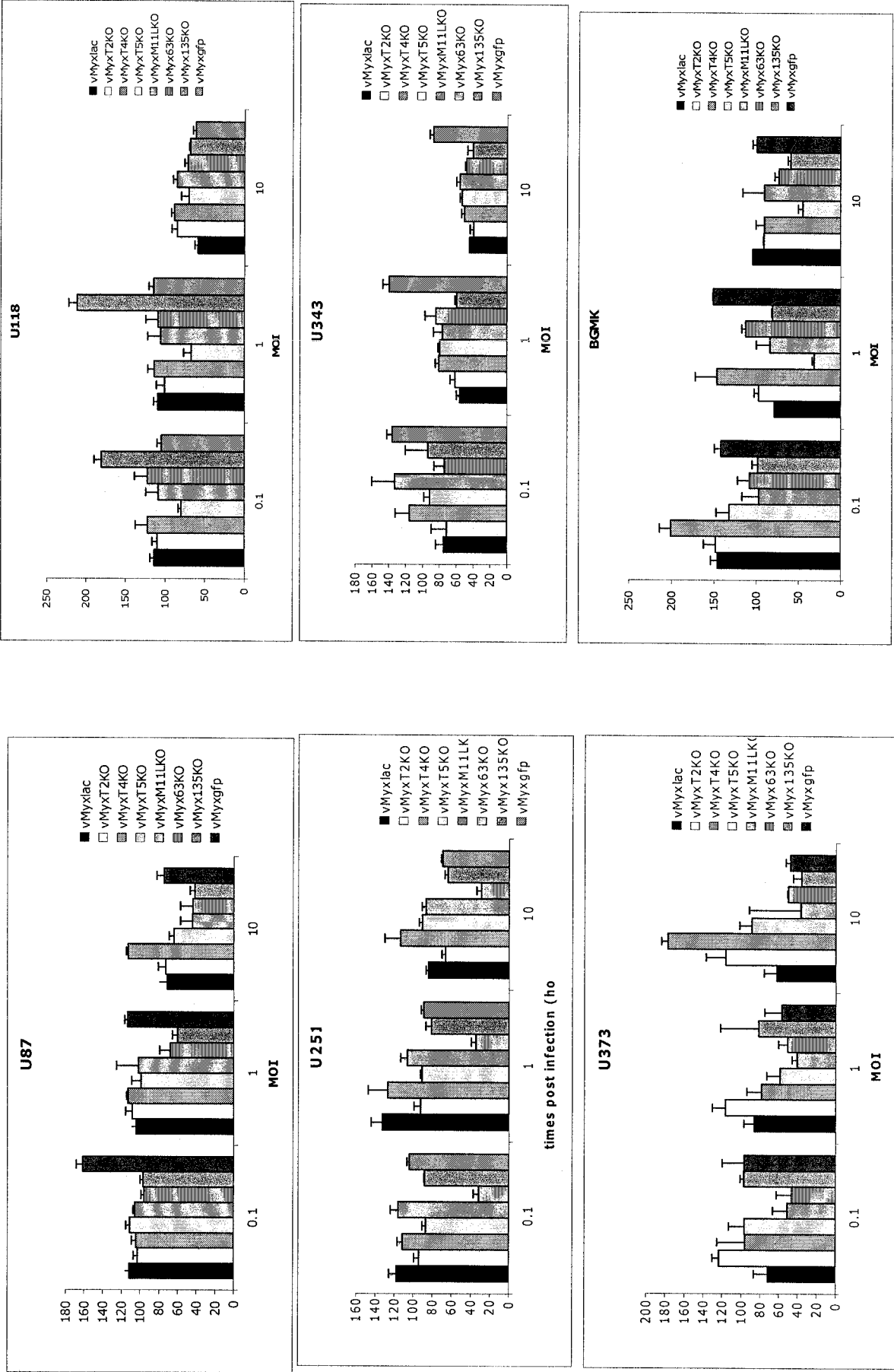


FIGURE 6

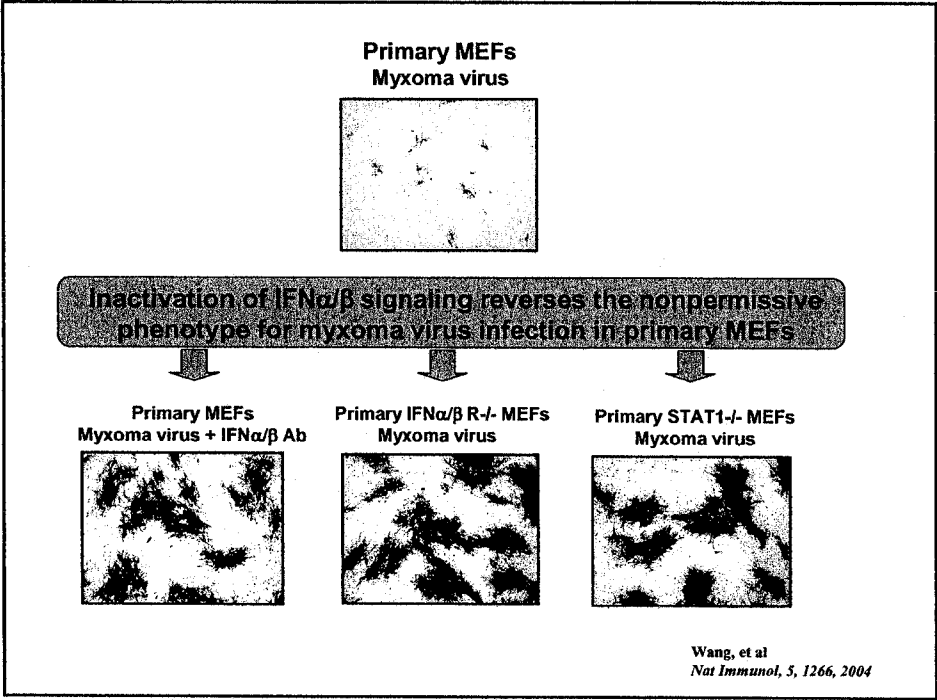


FIGURE 7

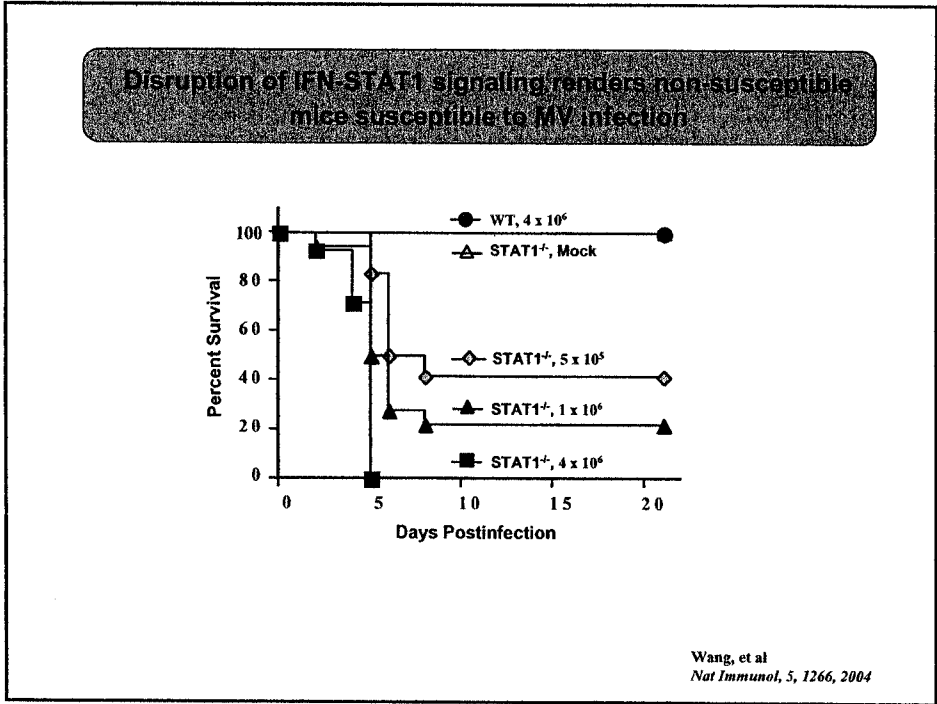


FIGURE 8

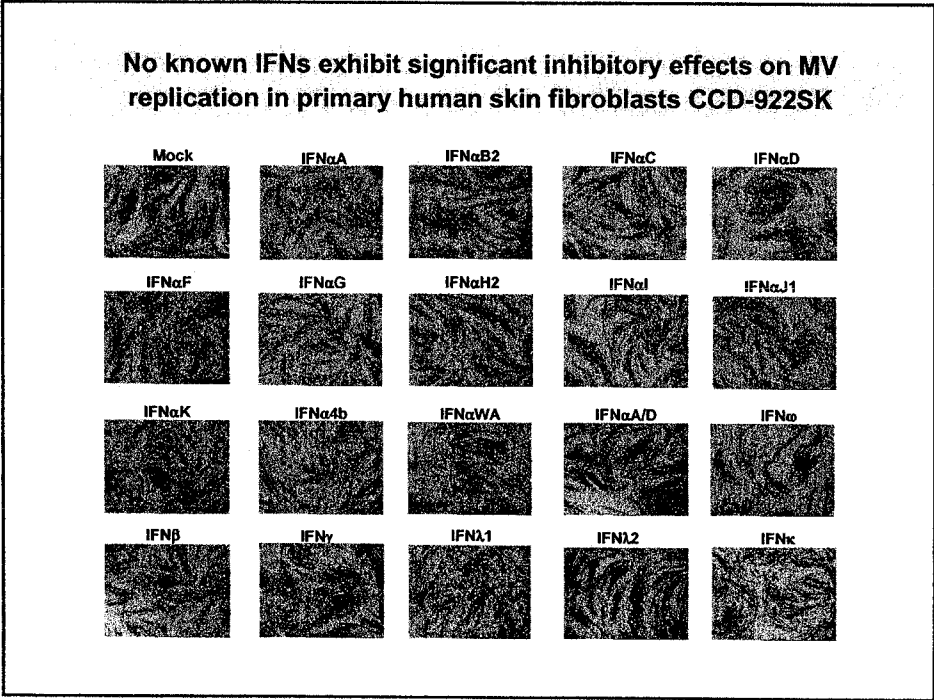


FIGURE 9

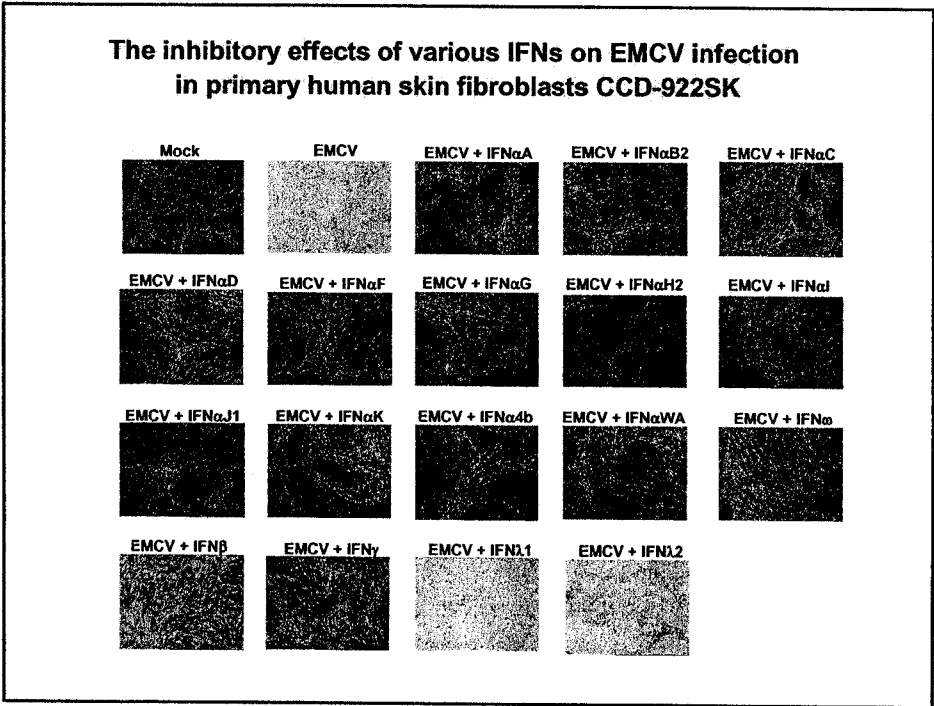


FIGURE 10

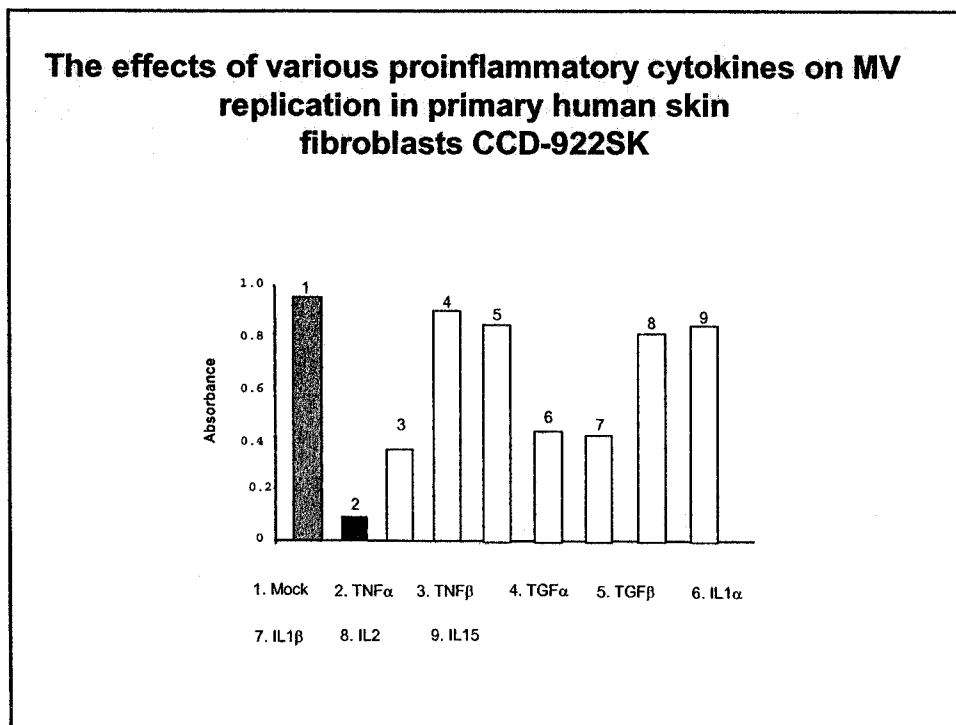


FIGURE 11

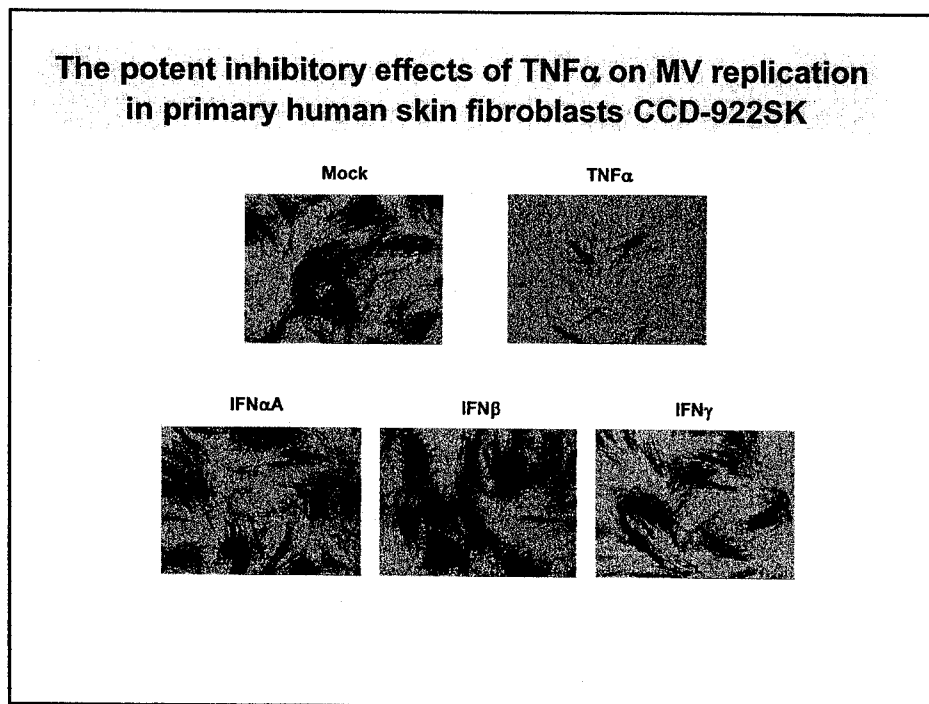


FIGURE 12

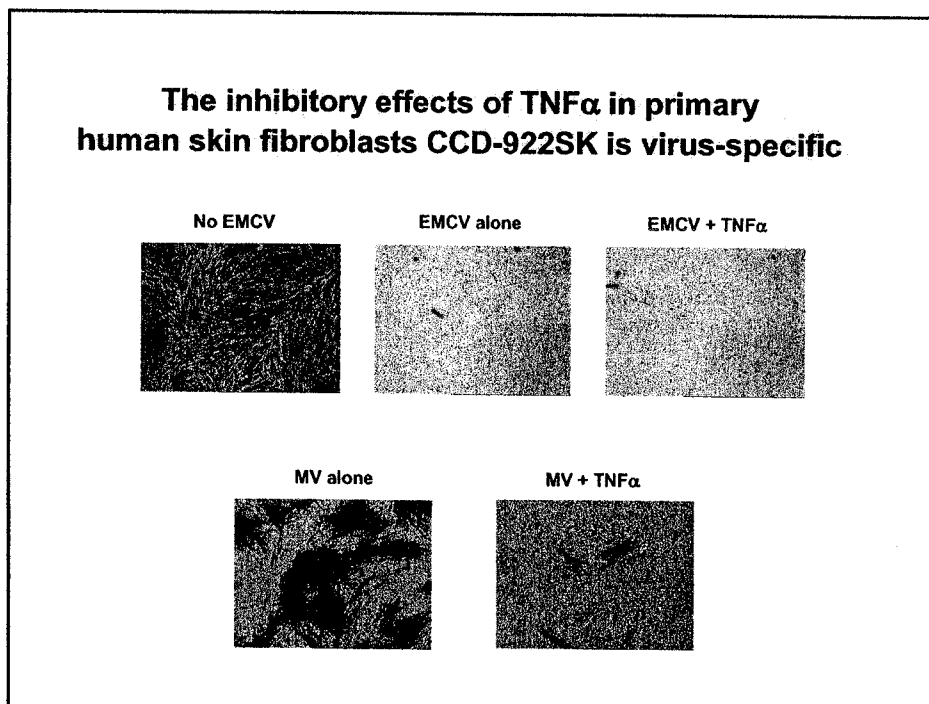


FIGURE 13

The construction of recombinant myxoma virus expressing human TNF α (1)

Step 1: PCR synthesis of flanking sequences for homologous recombination.

The left flanking sequence (FL) for insertion into myxoma SOD ORF (M131R) was PCR'd with primer pair:

FLF_KpnI: GGTACCATACGACGTCGTACGCGAATCTGGCA

FLR_MCS (multi-cloning site):

AAGATCTAAGTCGACCCCGGTAATGAATTCTACGTAGCGGCCGCAAGCTAG

CTCTTTATTAACTCGTAATAGCGAGGA (red colour indicating MCS)

The right flanking sequence (FR) was made similarly with primer pair:

FRF_MCS:

AGCTAGCTTGCGGCCGCTACGTAGAATTCATTACCCGGGGTCGACTTAGATCT

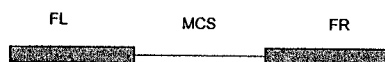
TAACCGGTGATCGATCCATTATAGGA (red colour indicating MCS)

FRR_SacI: GAGCTCTAGTATACAGCGATACGTCAATGGACA

FIGURE 14

The construction of recombinant myxoma virus expressing human TNF α (2)

Then, the FL and FR PCR products were mixed and re-PCR'd again with primer pair FLF_KpnI and FRR_SacI, and cloned into bluescript between KpnI and SacI, and sequence was confirmed (no sequence error).



The MCS contains RE sites : NheI, NotI, EcoRI, SmaI, SalI, and BglII

FIGURE 15

The construction of recombinant myxoma virus expressing human TNF α (3)

Step 2: Insertion of p7.5+gpt into the BglII site.

Step 3: Insertion of EGFP or EGFP+hTNF- α between NheI and SmaI sites.

The cassette was cut from the constructs based on pJS5, EGFP and hTNF α was driven by the synthetic early and late promoter.

Standard protocol for making recombination poxviruses was used.

FIGURE 16

The replication of recombinant MV expressing human TNF α is highly restricted in primary human fibroblasts CCD-922Sk

CCD-922Sk/WT MV



CCD-922Sk/
recMV-TNF α



pRKC/
recMV-TNF α



FIGURE 17

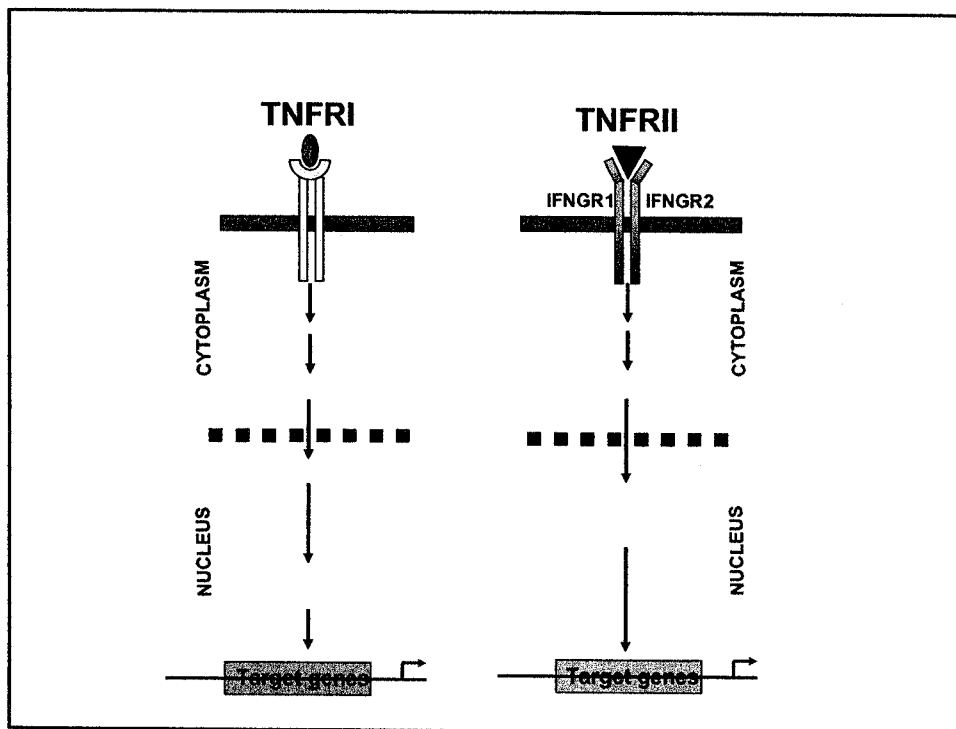


FIGURE 18

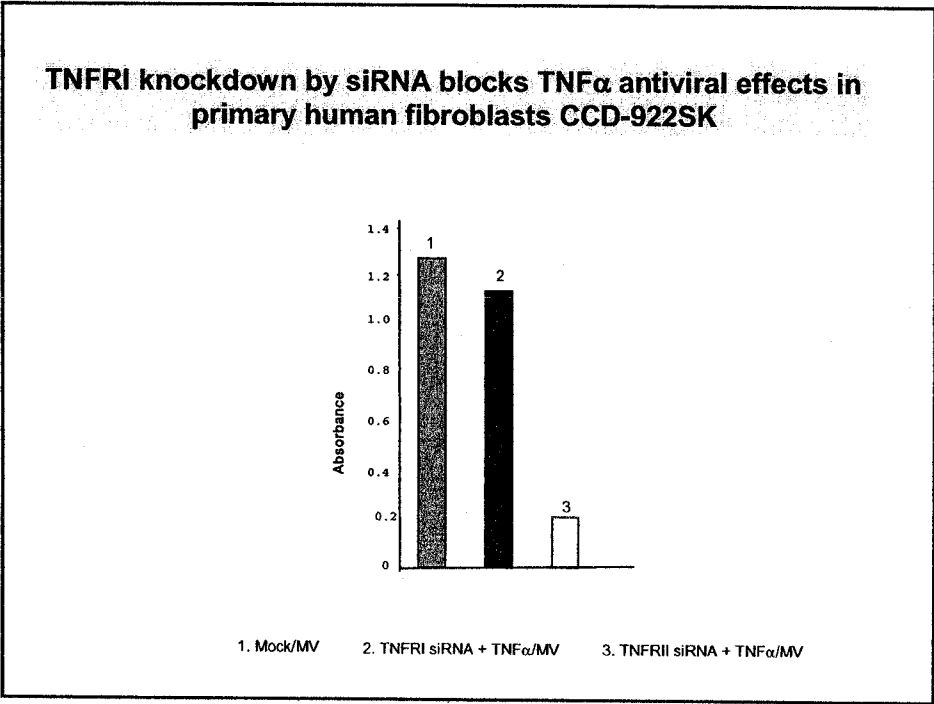


FIGURE 19

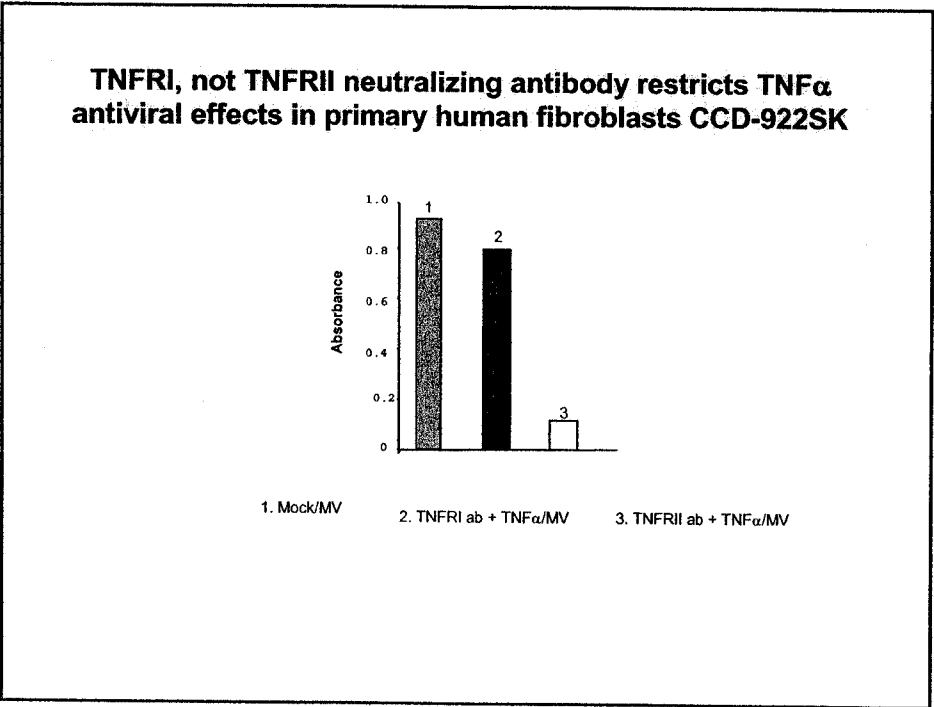


FIGURE 20

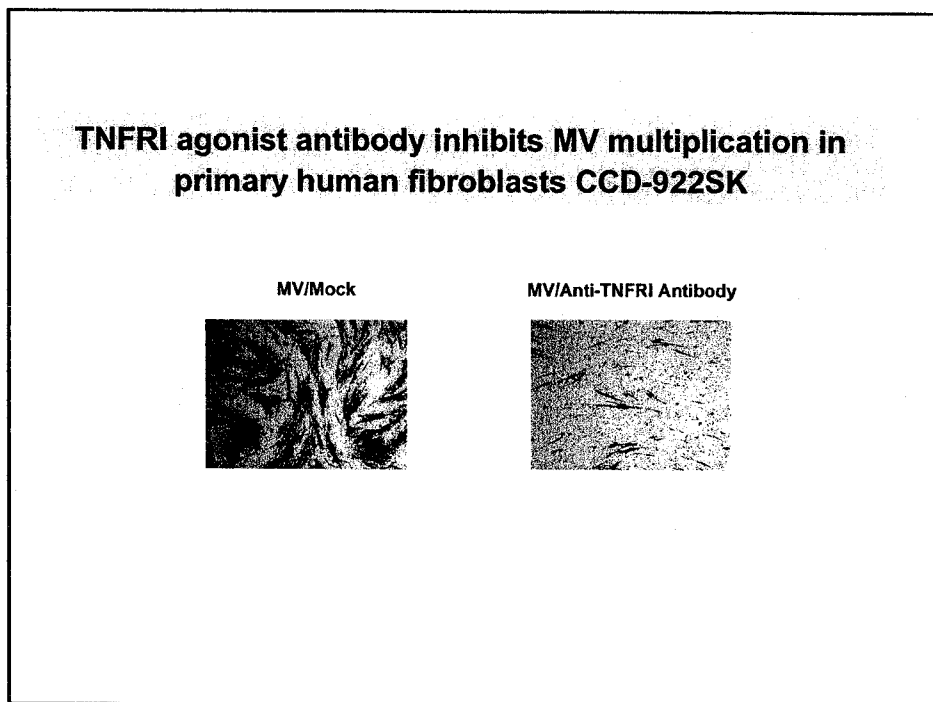


FIGURE 21

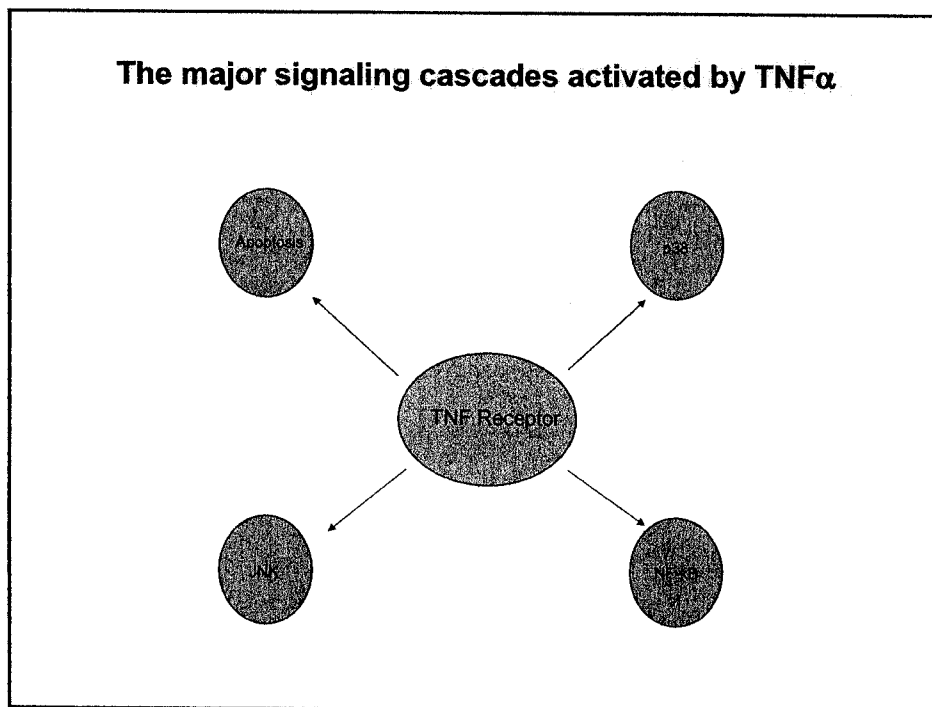


FIGURE 22

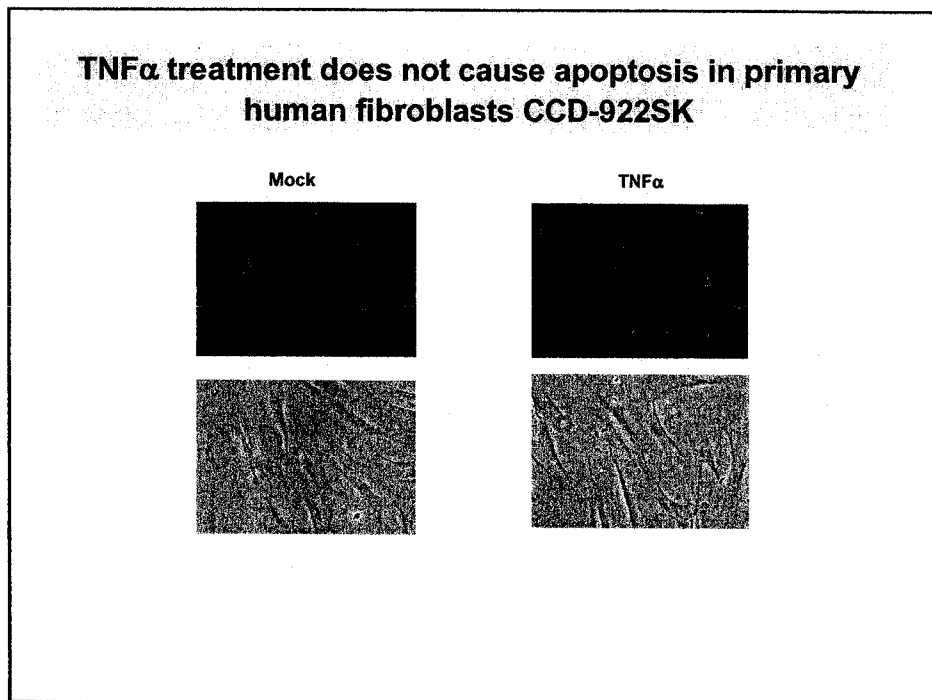


FIGURE 23

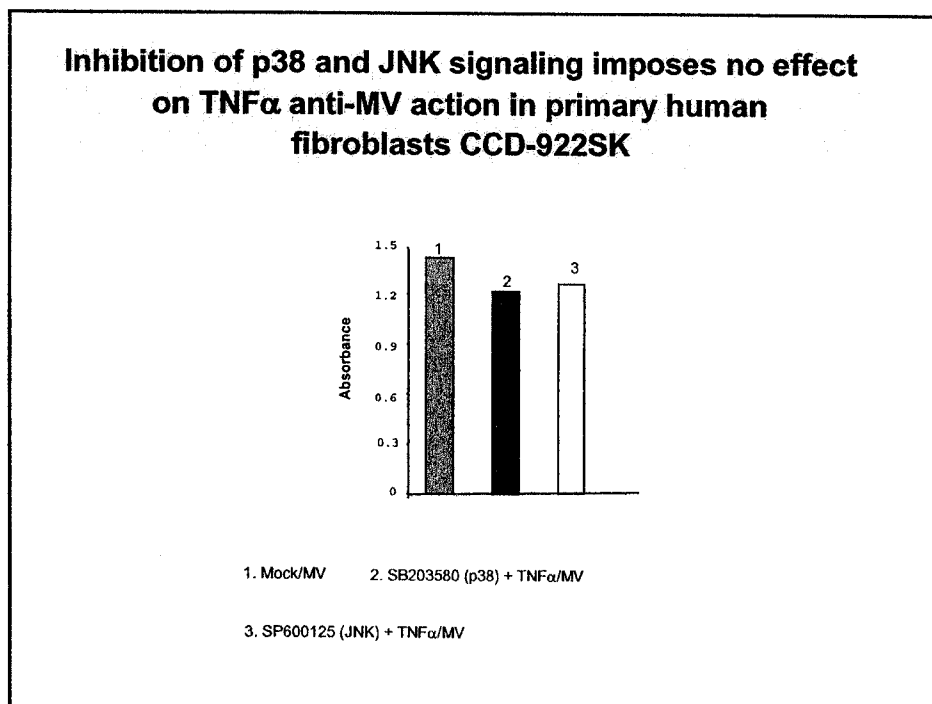


FIGURE 24

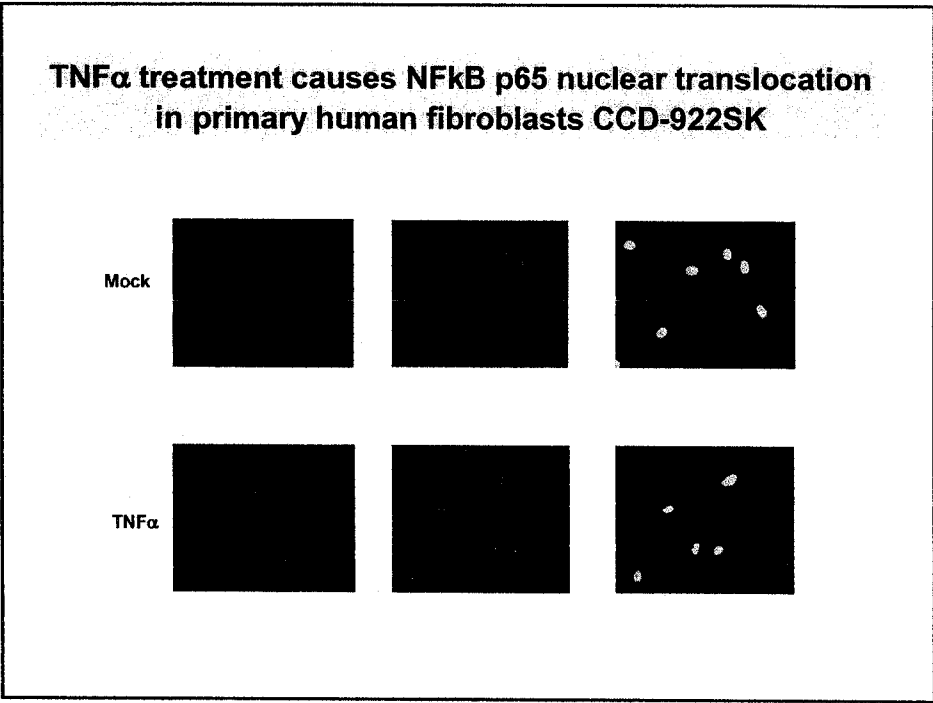


FIGURE 25

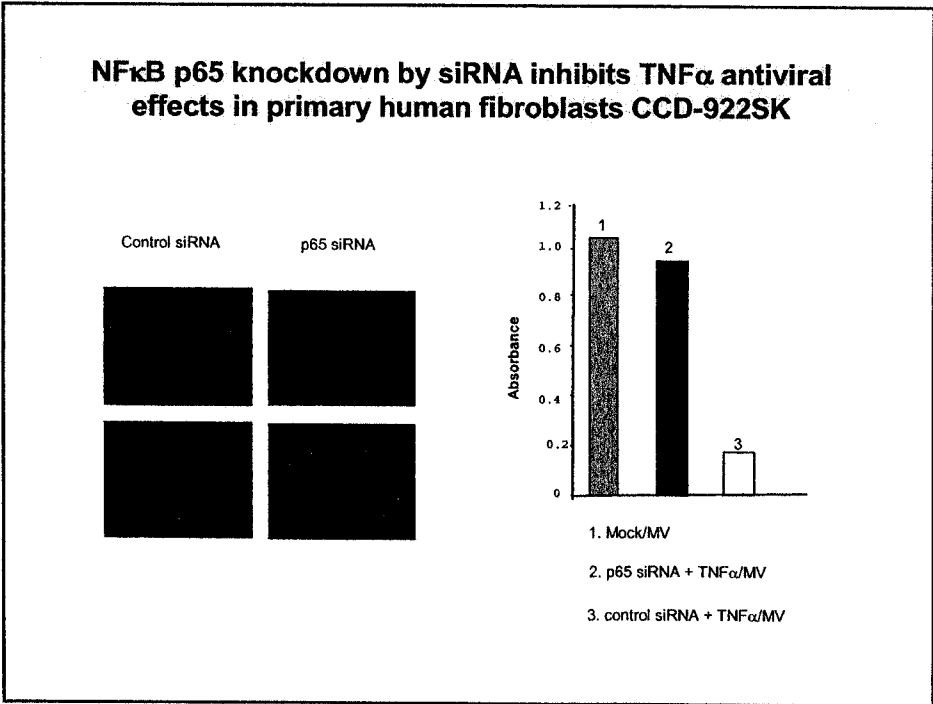


FIGURE 26

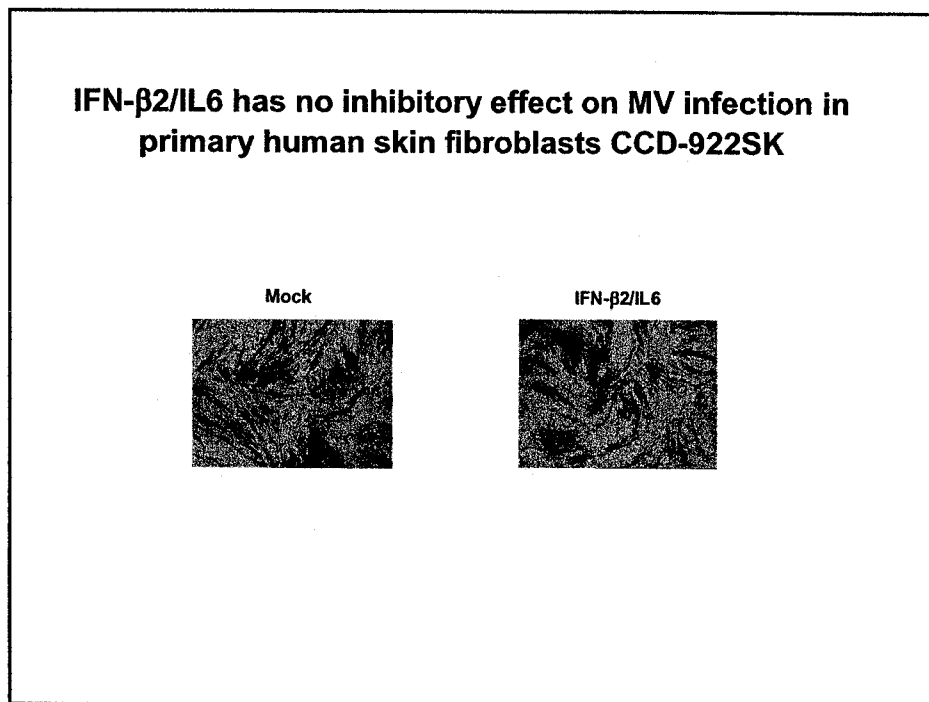


FIGURE 27

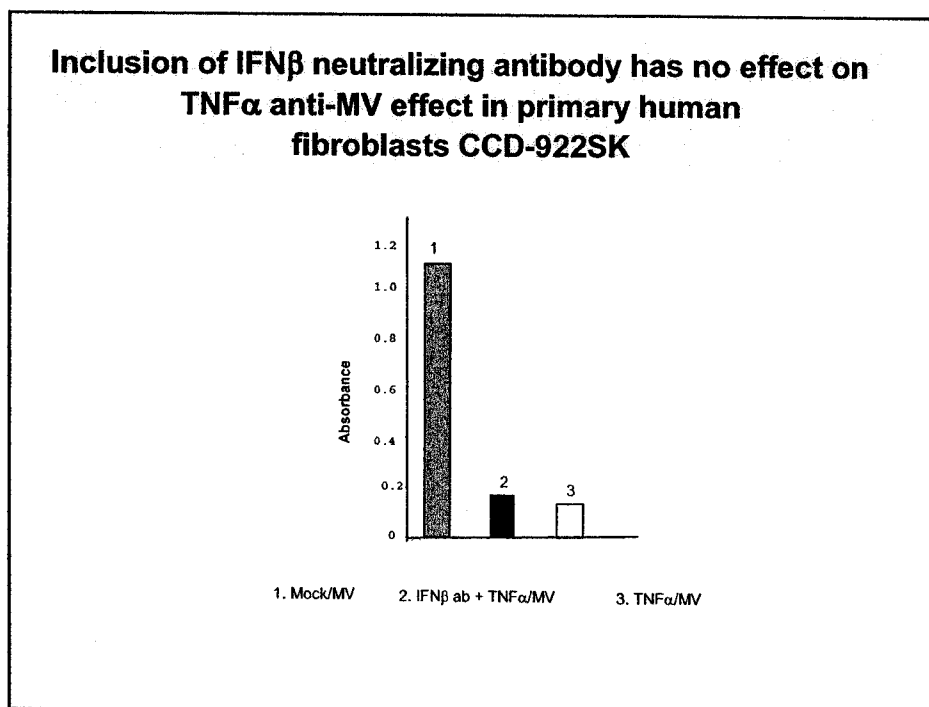


FIGURE 28

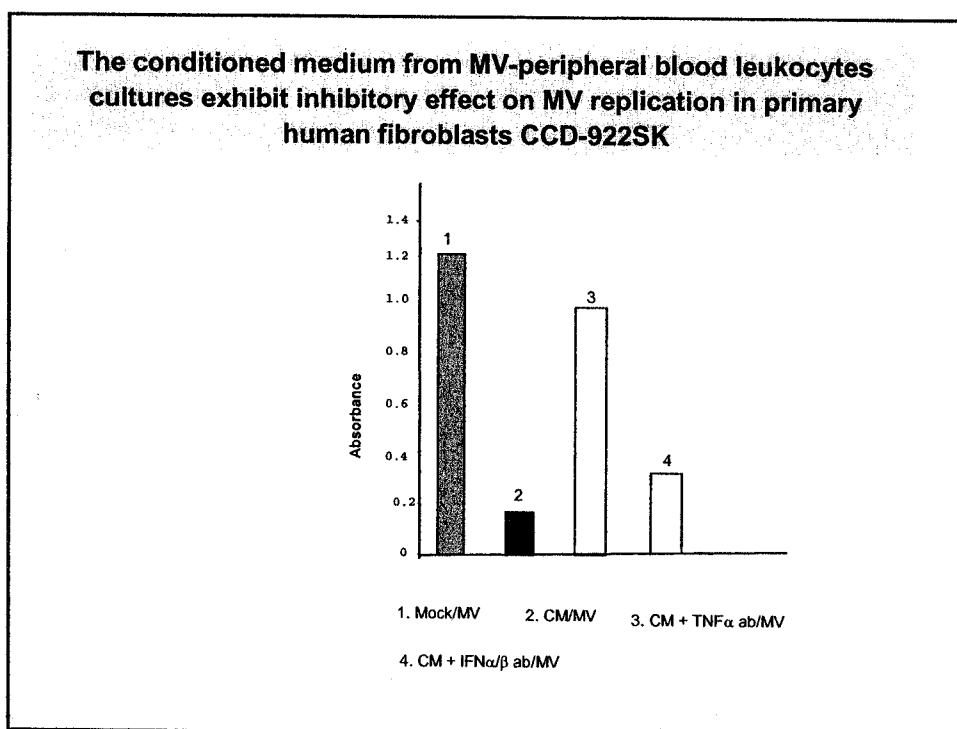


FIGURE 29

The antiviral effects of $\text{TNF}\alpha$ (and $\text{TNF}\beta$) on MV infection in primary human skin fibroblasts CCD-922SK

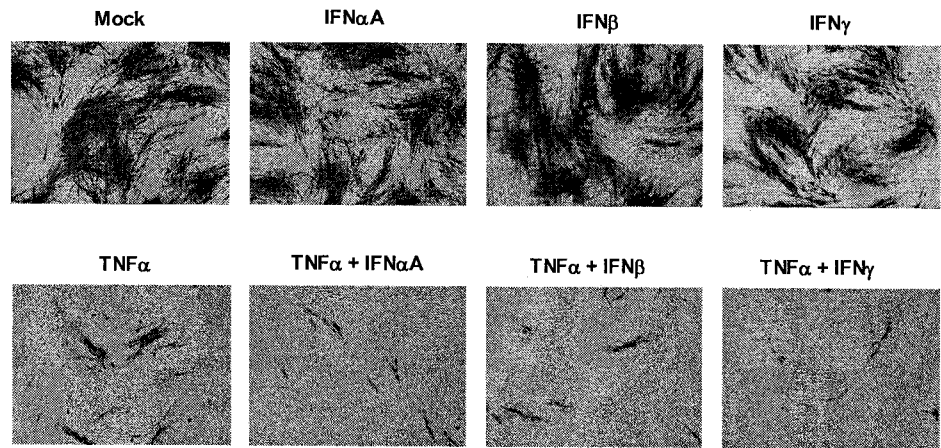


FIGURE 30

The impact of $\text{TNF}\alpha$ treatment on MV replication in human tumor cells

