



Review

Virokines: Mediators of Virus-Host Interaction and Future Immunomodulators in Medicine

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Abstract. A decade ago, after the discovery of the major secretory protein of vaccinia virus with structural similarity to complement control, the term virokinine was coined. The term virokinine, simply refers to a virally encoded secretory protein. During the past decade several virokines were discovered and most of them have been found to have immunomodulatory effects. A subset of virokines which resemble cytokine/chemokine receptors have been termed viroreceptors. Examples of viroreceptors include the TNF receptor homolog and the IL-1 β receptor homolog. During the past several years animal models have been developed to try to understand the *in vivo* role of the virokines. It has become evident from at least two studies that quite a few of the virokines down-regulate the inflammatory response elicited during infection. This down-regulation at least in one model system seems to indicate that it ensures the preservation of the viral habitat (site of infection). One obvious spinoff of the research on virokines is a new class of immunomodulators has become available as therapeutics in alleviating the symptoms of inflammatory disease conditions.

Key words: virokines; viroreceptors; virus-host interaction; virokinine therapeutics.

Complex DNA viruses like poxviruses encode for proteins that are required for viral replication and such proteins have been termed as essential proteins. For over a decade, it has been recognized that poxviruses also encode for additional proteins termed as non-essential proteins because they are not required for replication of the virus in cell culture. These so called non-essential proteins turned out to be very fascinating proteins because as we now know, they are involved in virus-host interaction. Vaccinia virus complement control protein (VCP) as it is now called contributed to the coining of the term “virokinine”. VCP is the first soluble microbial protein with proven immunomodulatory function. It has structural and functional similarity to the family of complement control proteins⁸. It has the greatest structural similarity to the human C4b-binding protein and the closest functional similarity to the first

human complement receptor, CR1. Because VCP consists of 4 short-consensus repeats (SCR) it belongs to the SCR-containing family which includes besides complement control proteins, IL-2 receptor and β_2 -microglobulin. Since the discovery of VCP, an amazing number of virokines have been discovered⁶ and their *in vitro* activities have been characterized and discussed here. Until recently there were no reports of animal models employed to understand the precise *in vivo* role of the virokines and this gave rise to skeptics, who felt that virokines were just some extra baggage that DNA viruses carried but that there was no real function that could be determined. As discussed below, this notion has changed and it has become evident that the virokines indeed play a vital role in controlling the influx of inflammatory cells and creating conditions favorable for viral growth. Lastly, at least 2 proteins have been

shown to have a clear therapeutic effect in alleviating inflammatory response. Thus, the age of viral modulators has arrived, akin to the age of antibiotics which began about 50 years ago.

The Repertoire of Virokines

The medium from poxvirus infected cells contains several virokines⁵, as shown in Fig. 1. Not all poxviruses encode all the known virokines. Cowpox virus is possibly the only virus with the most diverse and intact repertoire of known poxviral virokines¹². These include a complement control protein, termed as VCP or inflammation modulatory protein (IMP) that binds to

complement components, inhibits the formation of classical and alternate pathway convertase and causes cleavage of C3 in the presence of factor I¹⁰. The vaccinia virus growth factor (VGF) which can cause the increased proliferation of uninfected cells. The IL-1 β receptor homolog (vIL- β ^r) which can bind to IL-1 β and decrease the cellular influx. The TNF- α receptor homolog (vTNF^r) which can bind to the TNF and block TNF activity related functions. The chemokine binding protein (vCKBP) which binds to CC chemokines like MIP-1 α . The broad spectrum chemokine receptor binding activity found only in molluscum contagiosum and associated with the MC148 protein².

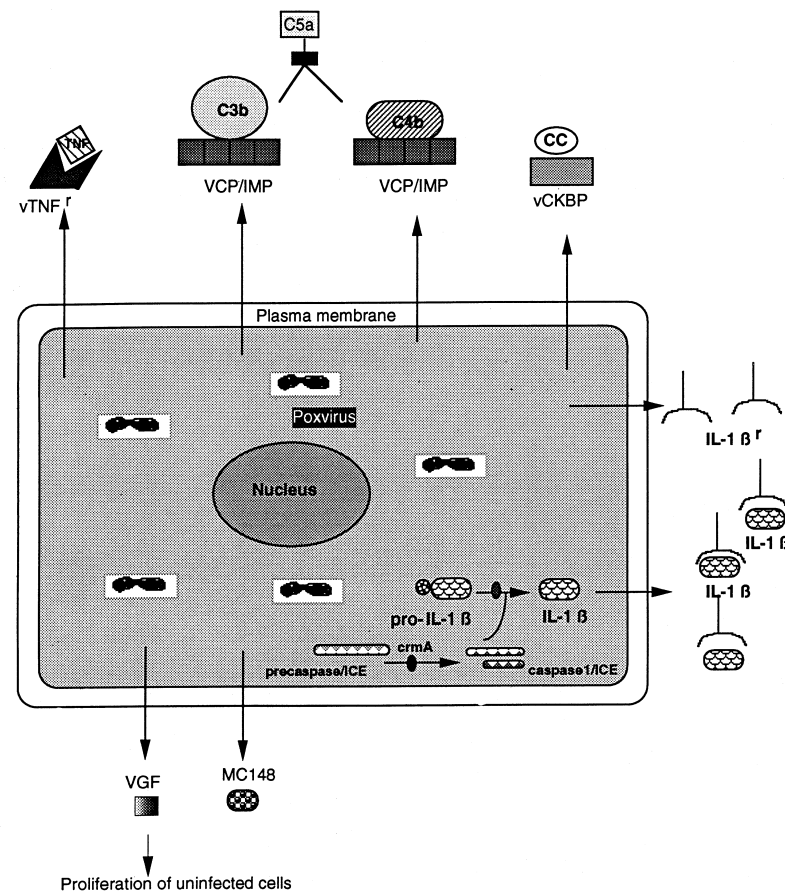


Fig. 1. Repertoire of virokines. The proteins which are secreted from poxvirus infected cells, also termed as virokines which bind to cytokines, complement components, chemokine receptor or which cause proliferation of uninfected cells are shown in the figure. Vaccinia virus complement control protein (VCP) or its homolog encoded by cowpox virus, termed the inflammation modulatory protein (IMP) consists of 4 short consensus repeats, characteristic of the family of human complement control proteins. The VCP and IMP can block both the classical and alternate pathway by binding to the third and fourth components of the complement system. This binding causes diminished generation of the chemotactic factors C3a and C5a, thereby reducing the influx of immune cells to site of infection. The vaccinia virus growth factor protein (VGF) causes increased proliferation of uninfected cells, resulting in the availability of greater number of host cells for viral replication. The MC148 is the chemokine-like protein secreted from molluscum contagiosum. The MC148 binds to both the α and β chemokine receptors but does not cause cellular activation, thereby acting as an antagonist of the α and β chemokines. The remaining virokines are cytokine/chemokine binding proteins and are termed the viroreceptors. The viroreceptors include the viral tumor necrosis factor receptor (vTNF^r), the chemokine binding protein (vCKBP) and the interleukin β receptor (IL-1 β ^r). In addition to binding to the IL-1 β , poxviruses produce a protein called cytokine response modifying protein (crmA) that can block both the biosynthesis of IL-1 β and the caspases, enzymes which mediate apoptosis, or programmed cell death

***In Vivo* Role of Virokines**

Two whole animal models have been developed in recent times to try to determine the precise outcome of the evasion of host defense by 2 different virokines. The first is a mouse model developed by my group in which congenic or knockout mice either low or lacking the complement components C5 or C3 or lacking MIP-1 α are injected with cowpox virus or cowpox virus (CPV) lacking the complement control protein, termed the inflammation modulatory protein. The viral injections are given subcutaneously in the footpad for quantitative measurement of the specific swelling response¹¹ or are given into an airpouch developed by injection of 1 cm³ of air in the back of the mouse, prior to injection of the viral preparation⁷. The airpouch technique is used to assess qualitatively, the type of cellular influx into the connective tissue lining the airpouch. The reason for selecting cowpox virus instead of vaccinia virus (a human virus) to perform our injections are numerous. CPV is a safe virus to use and was the first virus to be used as a vaccine against smallpox by Jenner. CPV is the most ancient virus with a wide host range which includes rodents. Our collaboration with the group led by Shchelkunov demonstrated that CPV has the most complete repertoire of open reading frames for immunomodulatory proteins. The reason we decided to use mice is because mice when injected in the footpads give a significant specific swelling response and the pathology can be easily followed by performing the histology on the tissues. Also, as stated earlier rodents are the primary reservoir hosts for CPV and therefore the infection is natural. Mice are available in a variety of strains and knockouts, allowing us to evaluate the influence of individual immune components in the pathogenesis of poxviruses. Mice also have a well-developed complement system, unlike the chick embryo system used earlier to study effects of individual gene deletions. Using this model system, we observed that in the absence of the IMP, there was a greater specific swelling response in BALB/c mice when injected in footpads¹¹ and also there was an increased mononuclear cell influx when injected in an airpouch model⁷. The effect was so dramatic that upon injection of the recombinant cowpox virus lacking IMP in the footpad model the foot was so swollen that it was almost about to fall off¹¹. In contrast, injection of BALB/c mice with the wild type CPV cause swelling that was almost half that of the recombinant virus lacking IMP. In the airpouch model the injection of CPV lacking IMP not only caused greater cellular influx but also resulted in a granulomatous lesion in the skeletal

muscle tissue surrounding the connective tissue⁷. This result was interpreted as the IMP contributing to preservation of viral habitat (infected cells and surrounding uninfected cells)⁴. This may seem like a new concept, but viruses do exert control over the region surrounding the infected cells and viruses seem to protect the uninfected cells to allow the growth of their progeny virus. This concept of viral habitat and its preservation by viral protein extends beyond virokines. Intracellular viral proteins like cytokine response modifying protein (crmA) of cowpox virus is able to block 3 events, the processing of the precursor of IL-1 β (a pro-inflammatory cytokine) by interleukin converting enzyme (ICE), the blockage of the processing of caspases like ICE to block apoptosis and the blockage of granzymes to block cell lysis. Thus viruses have evolved both intracellular and extracellular mechanisms to preserve viral habitat⁶.

The second animal model to study virokines has been developed by the McFadden group. They have used the myxoma virus a natural pathogen of rabbits and have demonstrated that indeed the lack of the viral chemokine binding protein (vCKBP) of myxoma virus resulted in the greater influx of macrophages in the injected skin tissue³.

Virokines and Live Vaccines

The *in vivo* study of virokines combined with the sequence information of the orthopoxviruses suggests that the relatively harmless CPV has a greater repertoire of immunomodulatory proteins than the variola virus which is a causative agent of smallpox and has caused millions of deaths. This could be interpreted as variola being more virulent because it has fewer immunomodulatory proteins. This taken together with our *in vivo* studies in which the virus lacking IMP is more virulent clearly indicates that in generating multivalent recombinant live vaccinia virus vaccines against heterologous microbial antigens, one will have to be very careful in not tinkering with the immunomodulatory function. Alternatively it would be advisable to generate a live vaccine using the modified vaccinia Ankara strain, which for some unknown reasons is highly attenuated and has been tested in humans.

Therapeutic Potential of Virokines

Virokines mimic their cellular homologs. What then is the advantage of using virokines over human proteins which they mimic. Firstly, virokine are significantly smaller than their cellular homologs. For instance VCP and IMP consist of 4 short-consensus repeats (SCRs)

while the human complement binding protein consist of 60 SCRs, distributed into 8 subunits of 4–8 SCRs arranged like a giant spider-like molecule. Secondly, virokines are much more potent than their cellular homologs. Again, VCP is almost 30 fold more potent than the same amount of human C4b-binding⁵. Lastly since virokines were originally derived or stolen from their respective hosts they are not very immunogenic in their original hosts.

What kind of disease conditions could one expect virokines to be useful as therapeutics? So far the biggest potential is in the treatment of inflammatory conditions with virokines like those that block the inflammatory response like Alzheimer's disease, restonosis following angioplasty, multiple system organ failure, xenograft rejection, systemic lupus erythematosus, arthritis, ischemia following heart attacks etc. It is envisioned that the anti-apoptotic proteins would also have a significant potential. Although it is difficult to envision what it might be.

The most well-developed model suggesting future therapeutic potential is the one described by the McFadden group. This group investigated the use of a purified virokin, secreted serine proteinase inhibitor, SERP-1, to reduce plaque development/growth (atherosclerosis) after balloon angioplasty-mediated injury. SERP-1 in the rabbit atherosclerotic model is associated with a focal reduction in macrophage influx that follows injury due to balloon angioplasty and this in turn inhibits the atherosclerotic plaque development⁹.

My group has shown that the amyloid beta peptide arising from the aberrant biosynthesis of the amyloid precursor protein is capable of activating complement by both the alternative and classical pathways and this can be blocked by the virokin preparation¹.

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