MIF-like Activity in Non-stimulated and Virus Infected Cell Cultures

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ABSTRACT

Macrophage-migration inhibition factor (MIF) is a lymphocyte-derived substance which plays an important role in cell mediated immunity. Soluble factors containing MIF-like activity and produced by non-stimulated and virus-infected non-lymphoid cell cultures have also been reported. In the present study, a MIF-like factor was repeatedly detected in Buffalo green monkey kidney cells infected with mumps and herpes simplex virus type 1 (HSV-1) indicating that this substance is reproducible and can be stimulated by two viruses of widely varying groups. Wistar-38 (WI-38) cell cultures also increased production of this substance in response to mumps but not HSV-1 infection, indicating that the production of this factor is not necessarily induced by all viruses. A factor which stimulated the spread of macrophages was also found to be induced in WI-38 cells by both viruses, suggesting yet another substance produced by non-lymphoid cells in response to viral infection. The ability of non-stimulated WI-38 cells to produce MIF-like activity was also confirmed, and this factor could be further stimulated or opposed by viral infection.

Introduction

Substances produced by lymphoid and non-lymphoid cells, which modify the behavior of other cells and can be triggered by a variety of agents, have been termed cytokines.1,2,3 Macrophage migration inhibition factor (MIF), one of the most widely studied cytokines, has been found to be released by a number of non-lymphoid cell types6,10,14 as well as by lymphocytes.4,8 The inhibition factor produced by non-lymphoid cells has been said to have MIF-like activity since it is similar in many aspects to MIF but has not been proven identical.6,10

Supernatant fluids from African green monkey kidney (BGM) cells infected with mumps or Newcastle disease virus have been demonstrated to have MIF-like activity.6 This activity was not found in the supernatant fluids of uninfected BGM cell cultures. Alternatively, MIF-like activity has been observed in the supernatants of
uninfected cell cultures such as HeLa, hamster malignant brain tumor and human embryonic lung (WI-38). The purpose of this study was to determine the effects of viral infections on a cell line (WI-38) already producing MIF-like activity and to determine the effects of another clinically significant virus, herpes simplex type 1 (HSV-1), on non-lymphoid cell lines.

Materials and Methods

Cell Cultures

BGM and WI-38 cell lines* were used in this investigation. Both cell lines were maintained with Eagle's minimum essential medium (MEM) in Earle's balanced salt solution supplemented with one percent L-glutamine and 100 units of penicillin and 100 mcg of streptomycin (P-S) per ml. In addition, the MEM contained 0.5 percent newborn calf serum for BGM cells and 0.5 percent heat-inactivated fetal calf serum for the WI-38 cells. The cells were grown in 16 × 125 mm screw cap culture tubes and fed upon receipt with the maintenance medium described.

Virus Preparation

The ABC strain of mumps virus† was passed four times in both cell lines with the fourth passage used as the stock virus. Mumps virus was harvested on the fifth day of each passage. The stock virus was titered by hemadsorption on the fifth day following inoculation. The titer of the stock virus, calculated according to the method of Reed and Muench, was 10⁵ tissue culture infectious doses 50/0.1 ml (TCID₅₀) in BGM cells and 10⁴-5 TCID₅₀/0.1 ml in WI-38 cells. HSV-1 (a human corneal isolate from our laboratory) was passed four times in both cell lines and harvested when the cytopathogenic effect was at least three plus. The titer of the fourth harvest, or stock virus, was 10⁵ TCID₅₀/0.1 ml in both cell lines.

Inoculation of Cell Cultures

Three concentrations of mumps virus, 10, 100 and 1000 infectious units (IU), were inoculated into BGM cell cultures, incubated for one hour at 37°C and then fed with one ml of MEM. Each concentration was incubated for 24, 48 or 72 hours. The same procedure was repeated with WI-38 cells. HSV-1 was also diluted to 10, 100 and 1000 IU, inoculated into BGM and WI-38 cell cultures, fed with MEM and incubated for 24, 48 or 72 hours.

Filtration of Pools

A pool refers to cell culture fluid from a given cell line, inoculated with a single viral dilution and incubated for one of the specified time periods. Each pool was ultra-filtered with a 0.05 µm Swinnex filter to remove viral particles. Each filtrate was inoculated into cell cultures to determine sterility and then frozen at −70°C until assayed.

MIF Assays

Migration areas from 12 capillary tubes were measured for each pool. Controls consisted of media from uninoculated cell cultures incubated for the same time period. Fluids from uninoculated cell cultures (BGM and WI-38) were also assayed for background MIF-like activity using fresh MEM as the control. MIF assays were done according to David's method. Packed guinea pig macrophages were diluted to a 10 percent cell suspension in RPMI 1640 supplemented with P-S, one percent L-glutamine and 10 percent heat-inactivated guinea pig serum. Heat-inactivated guinea pig serum was also added to each pool to a final concentration of 15 percent. All chambers were filled with filtered viral pools or control fluids and incubated for 24 hours at 37°C. The

* Microbiological Associates.
† Courtesy of Dr. Maurice R. Hilleman, Merck Institute, West Point, PA.
migration index (MI) was calculated as follows:

\[ MI = \frac{\text{Mean area of migration of macrophages in fluids from viral infected cultures}}{\text{Mean area of migration of macrophages in control fluids}} \times 100 \]

Each mean was calculated from areas of migration of 12 capillary tubes for each pool or comparable control.

Viability Studies

Macrophage viability was determined by one of two methods: (1) after measurement of the area of migration, the migratory cells were recovered from the chambers and viability was ascertained by trypan blue exclusion; or (2) the chambers were returned to the incubator for an additional 48 hours to demonstrate reversibility of migration inhibition.

Confirmation of Peak MIF-Like Activity

The virus dilutions giving peak MIF-like activity were reinoculated into the appropriate line of cell cultures and incubated for the time periods giving the greatest inhibitory responses. The supernatant fluids were filtered and assayed for MIF-like activity as described.

Results

In table I are compared the MI's of pools derived from mumps and HSV-1 infected BGM and WI-38 cells at varying dilutions and time periods. P values less than 0.05 were considered statistically significant. In mumps infected BGM cells, a MI which varied by 26 or more from the control value of 100 was significantly different, whereas in HSV-1 infected BGM cells, a MI which varied by 31 or more was significantly different. Ten and 100 IU of mumps virus incubated for 48 hours induced a MIF-like activity which was significantly different from the controls. Peak MIF-like activity (MI = 57 ± 12.6) was exhibited in the pool derived from 10 IU of mumps incubated for 48 hours. The pools from 1000 IU of HSV-1 incubated for 48 hours and 100 IU incubated for 72 hours were found to induce a significant MIF-like activity with MI's of 56 ± 12.8 and 69 ± 13.6, respectively. Pools from uninoculated BGM cells did not produce a significant amount of MIF-like activity when fresh culture medium was used as a control (table II).

Uninoculated WI-38 cells produced a significant amount of MIF-like activity with MI's of 56 and 46 at 24 and 72 hours, respectively, using MEM which had been incubated for the same periods of time as the controls (table II). In mumps infected WI-38 cells, a MI which varied from the control by 25 or more was significantly different while pools from HSV-1 infected WI-38 cells had to vary by 21 or more to be significantly different. As seen in table I, pools from 10 and 1000 IU of mumps virus incubated for 48 hours contained increased MIF-like activity with the peak inhibition (MI = 61 ± 10.5) occurring in the 1000 IU pool. HSV-1 was not found to increase significantly the MIF-like activity in WI-38 cells. At 72 hours, however, pools from 10 IU of mumps and 100 and 1000 IU of HSV-1 had MI's which exceeded the control by a significant amount (137 ± 15.1, 123 ± 10.8 and 157 ± 12.7, respectively).

To determine reproducibility of results, dilutions giving peak MIF-like activity were reinoculated into cell cultures and incubated for the appropriate time periods. The filtered culture media were assayed for MIF-like activity as described previously. In table III it is shown that comparable MIF-like activity was induced in each case when the experiments were repeated. Pools derived from the BGM cell line inoculated with 10 IU of mumps and incubated for 48 hours had
TABLE I
Comparison of Migration Indices* (MI) of Pools Derived from Mumps Virus and HSV-1 Infected BGM and WI-38 Cell Cultures

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Infect. Units</th>
<th>MI of Mumps Virus Pools†</th>
<th>MI of HSV-1 Pools</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Hrs.</td>
<td>48 Hrs.</td>
<td>72 Hrs.</td>
</tr>
<tr>
<td>BGM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>107 ± 15.9</td>
<td>57 ± 12.6*</td>
<td>86 ± 14.3</td>
</tr>
<tr>
<td>100</td>
<td>104 ± 15.7</td>
<td>71 ± 13.3f</td>
<td>76 ± 13.7f</td>
</tr>
<tr>
<td>1,000</td>
<td>76 ± 13.7</td>
<td>87 ± 14.4</td>
<td>74 ± 13.5%</td>
</tr>
<tr>
<td>Controls§</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>WI-38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>94 ± 12.3</td>
<td>73 ± 11.1%</td>
<td>137 ± 15.1%</td>
</tr>
<tr>
<td>100</td>
<td>89 ± 12.0</td>
<td>80 ± 11.4</td>
<td>127 ± 14.2</td>
</tr>
<tr>
<td>1,000</td>
<td>102 ± 12.8</td>
<td>61 ± 10.5%</td>
<td>103 ± 13.1</td>
</tr>
<tr>
<td>Controls§</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*Calculated as: mean area of test pool migration per mean area of uninfected control fluid x 100.
Mean is the average of 12 capillary tubes from three MIF determinations done in duplicate, each containing two capillary tubes.
†Pool is defined as: cell culture medium (MEM) pooled from one cell line inoculated with one viral concentration incubated for one time period.
§Controls consisted of culture media collected from the same lot number of uninoculated cell cultures which were incubated for the same time periods as the virus infected cell cultures.

Inhibition of macrophage migration was not due to cytotoxic effects on the cells. When the chambers which showed inhibition during the initial 24 hours were reincubated for an additional 48 hours, the MI’s equaled or exceeded the controls demonstrating a reversibility of migration inhibition which could only have been produced by viable cells. Also, when migratory cells recovered from chambers giving peak inhibition were compared to recovered control macrophages, viabilities were 71 percent and 70 percent, respectively, indicating that inhibition was not due to cytotoxic effects on the cells.

TABLE II
Background MIF-like Activity in Fluids from Uninoculated BGM and WI-38 Cell Cultures

<table>
<thead>
<tr>
<th>Time Hrs.</th>
<th>MI BGM</th>
<th>MI WI-38</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>94</td>
<td>56*</td>
</tr>
<tr>
<td>48</td>
<td>87</td>
<td>77</td>
</tr>
<tr>
<td>72</td>
<td>81</td>
<td>46*</td>
</tr>
<tr>
<td>MEM Control</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*Significantly different from control, P < 0.05

Discussion

Analysis of the MI’s in BGM cells shows that inoculation of this cell line with mumps induced a statistically significant MIF-like factor in three of the nine pools. Furthermore, two other pools (1000 IU at 24 hours and 100 IU at 72 hours) produced a substantial amount of MIF-like activity although not statistically significant. The peak MI of 57 was produced in the 10 IU pool incubated for 48 hours. This is in agreement with Flanagan et al6 who re-
ported a MI of 56.1 with mumps in BGM cells incubated for 48 hours.

When HSV-1 was inoculated into BGM cells, two of the nine pools tested showed a significant amount of MIF-like activity indicating that the MIF-like factor can be induced by at least one other virus, HSV-1, which belongs to a widely different group containing desoxyribonucleic acid (DNA) instead of ribonucleic acid (RNA) in its genome. Newcastle disease virus, a paramyxovirus, and SV40, a papova virus, have also induced MIF-like activity in BGM cells,1,6 further indicating the variety of viruses having this capability.

Mumps induction of MIF-like activity in cell cultures was not unique to the BGM cell line since mumps infected WI-38 cells also produced the MIF-like substance. Two of the nine pools tested contained significant MIF-like activity demonstrating an additional cell line capable of increasing production of MIF-like activity when infected with mumps virus. WI-38 cells infected with HSV-1 did not produce inhibitory factor in any significant amounts indicating that the production of this factor is not enhanced by all viruses. In vivo induction of MIF-like activity has also been demonstrated in extracts of parotid glands from monkeys infected with mumps18 and in spleen cells from mice inoculated intraperitoneally with mumps and other viruses.15

Production of MIF-like substances in unstimulated WI-38 cells has been reported in at least two other studies.10,14 In the present study the MI's of un inoculated cell cultures were compared with growth medium (MEM) and showed that BGM cells did not produce significant amounts of background MIF-like activity, which is in agreement with the work of Flanagan et al,6 whereas significant background MIF-like activity was exhibited in WI-38 cell cultures. Addition of mumps virus to WI-38 cell cultures enhanced migration inhibition of macrophages, whereas addition of HSV-1 did not.

While no physicochemical characterizations of the inhibitory factor were done in the present study, it appears that tissues of different stem cell origin may be capable of producing MIF or MIF-like substances whose effects are not dissimilar. Since this factor apparently has also been produced in vivo by non-lymphoid cells (parotid glands) in response to mumps virus infection in monkeys,18 this MIF-like substance may represent, among other things, another non-specific antiviral factor such as interferon. Production of macrophage inhibitory factors by simian virus (SV40), polyoma and Rous

### TABLE III

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Infect. Units</th>
<th>Mumps Humps 48 Hrs.</th>
<th>HSV-1</th>
<th>48 Hrs.</th>
<th>72 Hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st MI</td>
<td>2nd MI</td>
<td>1st MI</td>
<td>2nd MI</td>
</tr>
<tr>
<td>BGM</td>
<td>10</td>
<td>57</td>
<td>59</td>
<td>--</td>
<td>--</td>
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<tr>
<td></td>
<td>100</td>
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<tr>
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<td>Controls</td>
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<td>100</td>
</tr>
<tr>
<td>WI-38</td>
<td>10</td>
<td>73</td>
<td>74</td>
<td>--</td>
<td>--</td>
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<td></td>
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<td></td>
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<td>61</td>
<td>76</td>
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<tr>
<td></td>
<td>Controls</td>
<td>100</td>
<td>100</td>
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</tbody>
</table>
sarcoma viruses in virus-transformed lymphoid cells has been reported by Poste et al.\textsuperscript{11} Yoshida et al\textsuperscript{17} demonstrated that a viral induced MIF-like factor in SV40 infected non-lymphoid cell cultures, African green monkey kidney cells, shared in vivo and in vitro properties with lymphocyte-derived MIF. Whether there is a single MIF molecule or several different molecules with similar activity remains to be seen. This migration inhibitory substance, which can be produced by a variety of stimulated or non-stimulated cell lines, may indicate a more universal or diverse role for this factor.

Under certain conditions a migration stimulating factor (MSF) was noted. This factor was found only in WI-38 cells, occurred only at later incubation periods (72 hours) and was induced by both mumps and HSV-1. MSF has been described by several investigators as being induced by tuberculin\textsuperscript{9} and Brucella antigen.\textsuperscript{13} It has also been found in fetal calf serum\textsuperscript{7} and, as shown in this study, is induced by viruses in WI-38 cells. The biological significance of this stimulatory substance awaits clarification, but it is interesting to note that both inhibitory and stimulatory activity can be produced by the same cell line in response to viruses.

References


