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Human Fibroblast Interferon in Tears of Patients with Picornavirus Epidemic Conjunctivitis

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We report the levels of coxsackievirus type A24 (CA24) and the levels and type of interferon produced early during naturally acquired picornavirus epidemic conjunctivitis. Virus levels ranging from $10^{1.8}$ to $10^{5.8}$ 50% tissue culture infective doses per ml were detected in 29 of 37 acute (collected 1 to 4 days after onset of conjunctivitis) tear samples. Interferon ($10^{1.5}$ to $10^{3.3}$ U/ml) was detected in 12 of 29 tear samples collected on day 1, in 2 of 6 tear samples collected on day 2, and in 1 tear sample collected on day 3 after onset of conjunctivitis. The interferon activity in pooled tear samples was completely neutralized by antiserum against human fibroblast interferon, stable at pH 2.0, and active against different viruses. In addition, the interferon activity in tears, like human fibroblast and leukocyte interferons produced in vitro, protected human and rabbit, but not mouse, cells. This is the first report of production and identification of the antigenic type of interferon induced by an enterovirus during natural infection. The early appearance of fibroblast interferon suggests that it may be an important host defense at the local site of implantation against this and possibly other enterovirus infections.

Interferons can be induced in epithelial, fibroblast, and lymphoid cells in vitro and in vivo in response to a number of inducing agents, such as tumor cells, viruses, bacteria, rickettsiae, fungi, protozoa, biological products, and chemicals (1, 4-6, 8, 10, 16-22, 24-26, 28-33), and during suspected immunopathologically induced diseases (12). These human interferons have been classified as fibroblast, leukocyte, or immune interferon by their antigenic, physicochemical, and biological properties (17, 21). Taken together, these studies help support the developing concept that with the development of more definitive methods of determining interferon types, the type(s) of interferon produced during a disease may be used as a marker to identify various components of the infectious process(es). For example, leukocyte interferon is produced by nonsensitized leukocytes in vitro in response to foreign cells (2, 28) or virus infection (11, 24, 33), and acid-unstable (immune) interferon has been reported in patients with suspected autoimmune diseases (12). In this report, we studied virus levels and the levels and type of interferon present at the site of local infection in patients with picornavirus epidemic conjunctivitis (PEC) caused by coxsackievirus A24 (CA24) (34).

The studies reported suggest that fibroblast interferon is probably the only interferon produced early in response to local enterovirus replication in the cells of the conjunctiva and cornea and that this interferon may be an important host defense against this and possibly other enterovirus infections.

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MATERIALS AND METHODS

Tear specimens. An acute tear sample (10 to 70 μl) was collected 1 to 4 days after onset of illness in capillary pipettes from servicemen having PEC caused by coxsackievirus A24 (CA24). The collection method was the same as described previously by Yin-Murphy et al. (34).

Virus quantification. Tears were diluted in Eagle minimum essential medium containing Earles salts supplemented with 2% fetal bovine serum and antibiotics (100 U of penicillin, 100 μg of streptomycin, and 100 μg of gentamicin per ml). The 50% tissue culture infective dose of virus was determined in human foreskin (HFS_4) fibroblast cultures as described previously (15).

Quantification of interferon. The amount of antiviral activity in tears was determined on human amnionic WISH cells (American Type Culture Collection), mouse L-cells, and rabbit kidney (RK_{13}) cells by using a 50% cytopathogenic reduction microassay and Sindbis virus as challenge (27) or a 50% plaque reduction microassay with vesicular stomatitis virus as challenge (9). Wild-type virus was not removed from the tear samples since it did not inhibit the replication of Sindbis or vesicular stomatitis virus (15).

Interferons and antisera to interferons. Human leukocyte and fibroblast interferons (National Institutes of Health reference standards) and rabbit anti-

human leukocyte interferon and anti-human fibroblast interferon antisera were obtained from the Antiviral Substances Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Staphylococcal enterotoxin A (T-cell mitogen)-induced human immune interferon was obtained as previously described (14). Immune interferon titers are expressed in terms of a reconstituted lyophilized laboratory human immune interferon standard containing $10^{3.0}$ U of antiviral activity per ml. Leukocyte and fibroblast interferon titers are expressed in terms of their respective National Institutes of Health reference interferons.

Characterization of interferon activity in tears. Pools of tear samples consisted of 10 μl of tear sample from four patients (each sample contained ≥100 U of interferon per ml). All tears for characterization studies were collected on day 1 or 2 after onset of illness. The tear pools and control interferons were assayed for antiviral activity on human WISH, rabbit RK_{13}, and mouse L-cells (see above) to determine the cell species specificity. Replicate cultures were challenged with Sindbis or vesicular stomatitis virus to determine virus specificity. Pooled tears and control interferons were incubated at pH 2.0 for 24 h to determine acid stability and mixed with equal volumes of medium containing 100 U of anti-human leukocyte or anti-human fibroblast interferon for 2 h at room temperature to determine the interferon type. Residual interferon activity was assayed on WISH cells, and the results are expressed as a percentage of the respective control.

RESULTS

Levels of virus and interferon in tears of PEC patients. The levels of CA24 and interferon in tear samples collected 1 to 4 days (acute tear sample) after onset of conjunctivitis are shown in Table 1. Interferon ($10^{1.5}$ to $10^{3.3}$ U/ml) was detected in 15 of 37 tear samples collected 1 to 4 days after onset of illness. The levels of virus ranged from $10^{1.8}$ to $10^{5.8}$ 50% tissue culture infective doses per ml in the 15 tear samples which contained interferon, whereas the virus levels in the 22 tear samples that did not contain interferon ranged from $<10^{2.0}$ (8 samples) to $10^{4.5}$ 50% tissue culture infective doses per ml. Of six tear samples collected on day 2 after onset of conjunctivitis, two contained interferon and virus, two contained virus only, and two did not contain either virus or interferon. The day 3 sample contained interferon and virus, and the day 4 sample contained only virus. There was no significant correlation between the level of interferon and the level of virus in tears and the day after onset of illness with respect to tears collected on day 1 or 2 after onset of PEC. There were not enough samples collected on days 3 and 4 to test such an association. However, interferon was not detected in tear samples that did not contain virus. No other viruses were isolated.

Characterization of interferon in tears of PEC patients. To establish the type(s) of interferon present in pooled tear samples, specific anti-human fibroblast interferon and anti-human leukocyte interferon sera and physicochemical and biological properties were used (Table 2). The interferon in two pools of tear samples (each pool consisting of tears collected 1 or 2

TABLE 1. *Levels of virus and interferon in tear samples from PEC patients*

Day after onset of illness	Patient no.	Interferon (log_{10} unit per ml)	CA24 (log_{10} $TCID_{50}$[a] per ml)
1	1	3.3	5.8
	2	2.8	4.8
	3	2.5	4.8
	4	3.0	4.3
	5	2.5	4.3
	6	2.8	3.3
	7	1.5	3.2
	8	2.3	2.6
	9	3.0	2.4
	10	1.8	2.4
	11	3.0	2.1
	12	2.5	2.0
	13–29	<1.5	<2.0–4.5[b]
2	30	2.5	3.8
	31	2.0	1.8
	32–35	<1.5	<2.0–3.9
3	36	2.5	3.8
4	37	<1.5	3.3

[a] $TCID_{50}$, Fifty percent tissue culture infective dose.
[b] Range of virus in tear samples which did not contain interferon.

TABLE 2. *Antigenic characterization of the interferon in tear samples of PEC patients*[a]

Sample	Interferon activity (%) remaining after treatment with antiserum to:	
	LeIF	FbIF
Pool A	100	<10
Pool B	100	<10
FbIF	100	<10
LeIF	<10	90
ImIF	100	100

[a] Pool A was composed of tear samples collected on day 1 after onset of conjunctivitis. Pool B was composed of tear samples collected on day 1 or 2 after onset of conjunctivitis. Each pool contained between 100 and 300 U of interferon activity. Control interferons and pooled interferon samples were mixed with a dilution of the antiserum which contained 100 U of anti-interferon activity (enough anti-interferon activity to neutralize 1,000 U of interferon). Abbreviations: LeIF, human leukocyte interferon; FbIF, human fibroblast interferon; ImIF, human immune interferon.

days after onset of conjunctivitis from four patients) was neutralized to undetectable levels (<10 U) by antiserum to human fibroblast interferon. The interferon in tears was similar to fibroblast and leukocyte interferons since it was stable at pH 2.0 for 24 h, active against different viruses, and not active on mouse cells (17, 21). In addition, the interferon in tears was different from immune interferon since it protected rabbit cells (6, 17, 21). Thus, the interferon in tears was antigenically, biologically, and physicochemically similar to fibroblast interferon produced in vitro.

DISCUSSION

Antiviral activity was detected in 15 of 37 (38%) acute tear samples collected from patients having PEC caused by CA24. It was detected in 12 of 29 tear samples collected on day 1 and in 2 of 6 tear samples collected on day 2 after onset of conjunctivitis. The antiviral activity found in tears was demonstrated to be interferon by its activity against both vesicular stomatitis virus and Sindbis virus, by its restricted activity in mouse cells, and by its acid stability. The interferon was similar to fibroblast and leukocyte interferons since it was active on rabbit kidney cells (6, 17, 21). Further, the interferon activity in tears was neutralized to undetectable levels (<10 U) by antiserum to fibroblast interferon. These findings suggest that all of the detectable interferon in the tears of these patients was antigenically, physicochemically, and biologically similar to fibroblast interferon produced in vitro by virus-infected cells.

The finding of high levels of CA24, the association of interferon production with virus replication, and only fibroblast interferon production suggests that the early signs and symptoms associated with this local enterovirus infection of the eye are primarily due to virus implantation and replication in cells of the conjunctiva and cornea. If human leukocyte interferon had been found in tears, leukocytes infected with virus or leukocytes interacting with virus-altered cells (2, 11, 24, 28, 33) might be expected to be involved in the infectious process. The presence of human immune interferon might suggest an immunopathological process involving T lymphocytes (8, 12, 29). Mixtures of interferon types could suggest a combination of the different infectious and pathological processes. However, the detection of only fibroblast interferon suggests that CA24 infection is limited mainly to fibroblast and epithelial cells (cell types known to produce mostly fibroblast interferon) at the surface of the conjunctiva and cornea. This is consistent with the punctate lesions of PEC involving only the superficial layers of the cornea (13, 23) and high levels of virus early in infection (15). In addition, human Chang conjunctival cells and primary human conjunctival/corneal cells produce fibroblast interferon in vitro (personal observation). However, these findings do not rule out the production of other interferon types during the later stages of infection.

We and others have found that some enteroviruses induce low levels of interferon in vivo and in vitro (11, 21, 26, 30); however, they are generally considered to be very poor inducers of interferon. Thus, it was surprising to find moderately high levels of interferon during CA24 eye infections. However, interferon was detected in only 38% of the acute tear samples. This low frequency may be explained by our inability to detect interferon because (i) tears were collected from patients with mild to severe conjunctivitis at only one time during the course of active infection, (ii) interferon can be diluted by reflex tear fluids, (iii) interferon may be present at levels below the initial dilution (1:30) of the interferon assay, or all of these. In addition, interferon inducibility of the virus may vary from isolate to isolate, and individuals may vary in their ability to produce interferon. Some of these factors are being investigated at present since little is known about induction of interferon by enteroviruses in vivo, especially at the sites of implantation in humans.

It is known that intersitial levels of interferon may be much higher than those found in body fluids (7), that interferon-treated cells do not replicate viruses effectively, and that interferon-treated cells can transfer antiviral resistance to surrounding cells (3); thus, the early appearance of fibroblast interferon in tear samples suggests that fibroblast interferon may act as an early host defense against CA24 at the surface of the eye. This suggestion is supported in part by the in vitro observation that CA24 is sensitive to interferon (26). One might then suggest that the relatively benign nature of this infection may be due partly to local production of interferon or an early appearing anti-CA24 neutralizing activity (15) by the cells associated with the eye or both.

In summary, fibroblast interferon production at the site of infection suggests that the majority of the pathology associated with this enterovirus infection of the cells of the conjunctiva and cornea is directly related to virus replication and that interferon may play a role as a host defense during the early stages of infection.

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