Archives of Virology 73, 207—217 (1982)

Archives of Virology

Some Newly Recognized Aspects of Resistance Against and Recovery from Influenza

Brief Review

By

A. ENNIS

Department of Medicine,
University of Massachusetts, Worcester, Mass., U.S.A.

Accepted June 10, 1982

Introduction

During the course of influenza infection, inflammation of respiratory tract tissues occurs and the predominant cells in this inflammatory process are mononuclear cells (1). The nature of these cells and their role in the lung, adjoining lymph nodes, spleen or in the peripheral blood in the infectious process and recovery has not been defined. Progress has been made however since the observation by ZINKERNAGEL and DOHERTY (2) that a specific lymphocyte, the cytotoxic-T lymphocyte, recognized and killed virus infected target cells which possessed both the virus and the self antigens (H-2) in common with the virus antigen used to stimulate the lymphocyte and the self antigen of the killer lymphocyte. Specific killer cells, therefore, might be identified following influenza infection or immunization, and studies using these markers could be performed to evaluate the function of these cells. More recently, another effector lymphocyte has been described, the natural killer (NK) cell. These cells unlike the cytotoxic-T lymphocyte can be directly detected in the circulation of normal non-immune mice and humans, and their activity appears to be increased shortly after virus infection begins (3). In addition, their killing activity is not restricted by self antigens and is not virus specific. The purpose of this commentary is to briefly summarize some of the observations that have been made using techniques that measure the activity of these two types of killer lymphocytes in the course of influenza infection, and to incorporate the observations into an hypothesis of their possible roles in the prevention of or recovery from influenza.

Prevention of Influenza

There is a reasonable amount of information obtained primarily from experiments performed in animal models which suggests that resistance against infectious challenge with influenza virus can be correlated with the presence of

0304-8608/82/0073/0207/$ 02.20

antibody to the surface antigens of the virus used to challenge. Using infectious virus to immunize donor mice, FAZEKAS de St. Groth demonstrated that transfer of antibodies from the immune mice protected recipient mice against intranasal challenge. He noted that the level of protection correlated with the amount of antibody detected in local secretions at the time of challenge and that transfer of antibody from the same species was more effective than from another species (4). SCHULMAN reported a decrease in the pulmonary virus titer of mice which had been previously immunized by live virus infection with a virus which shared the surface neuraminidase of the challenge virus, but had an antigenically distinct hemagglutinin. The decrease in pulmonary virus titers in mice possessing immunity to the hemagglutinin was greater than that observed in mice with immunity only to the neuraminidase (5). VIRELEZIER (6), subsequently demonstrated that transfer of hyperimmune rabbit serum, which contained only antihemagglutinin antibody to the challenge virus, conferred significant protection against challenge to recipient mice if administered before or very soon after challenge. He failed to protect in similar experiments using antisera to the nucleoprotein or matrix protein. ENNIS *et al.* (7) reported that the passive transfer of antibody from mice given inactivated influenza vaccine protected recipient mice against death provided anti-hemagglutinin antibody was detectable in the circulation at the time of virus challenge, and that antibody was given up to twelve hours after virus challenge significant protection was not observed if antisera were given after 12 hours. In a series of experiments performed in ferrets by POTTER *et al.* (7), there was general agreement with the results obtained in mice, namely the presence of antibody in the circulation or in respiratory secretions appeared to be associated with significant protection against challenge.

There is much less information available about the mechanism of protection of humans exposed to challenge with influenza virus. It is known that previous infection with an identical strain of virus confers substantial immunity upon later challenge. This observation has been made during secondary waves of influenza and was very obviously demonstrated in 1977 when the 1950 (HINI) strain recirculated and caused no disease in those that had experienced infection with HINI virus strains in the 1950s, but caused illness in individuals aged less than twenty-five years. In addition, it has been generally accepted that high titers of antihemagglutinin inhibiting antibody are significantly associated with protection against a virus with a closely related hemagglutinin based on serological studies using the hemagglutinin inhibition test. HOBSON *et al.* (9) demonstrated this using sera obtained prospectively both before natural field epidemics and from human volunteers challenged under experimental conditions. There are no human data comparable to the mouse data using transfer experiments with hyperimmune sera to directly demonstrate that the antibody to the hemagglutinin was the protective factor.

The obvious interpretation of the protection mediated by passive transfer of serum containing antibodies to the hemagglutinin is that the antibody resulted in neutralization of at react some of the input virus before it multiplied and spread. It is probable that this passively transferred antibody would be most effective in the respiratory tract secretions of the recipients. It has been technically difficult to measure antiviral antibodies in respiratory secretions of experi-

mental animals and humans, but recently COUCH *et al.* (10) used a radioimmunoassay to measure antibodies in the respiratory tract secretions, and MURPHY *et al.* (11) have used a sensitive ELISA technique (11). It is interesting that COUCH *et al.* reported the best correlation between resistance to challenge and serum antibody levels of IgG (10), while MURPHY *et al.* observed it with IgA antibody in secretions (B. R. MURPHY and M. L. CLEMENTS, personal communication).

The information available from human studies therefore does not formally demonstrate that the protective mechanism of resistance to influenza challenge is antibody to the major surface glycoproteins especially the hemagglutinin. There is the possibility that in spite of the association between the presence of such antibodies in the sera and respiratory secretions with resistance to challenge, the antibodies are not the protective mechanism. Protection might be mediated at least partially by other host factors, for example, memory cytotoxic-T lymphocytes, which may be present in the challenged recipients. Immune induction of gamma interferon, although nonspecific in its effector function, would be induced only in immune individuals, and might therefore offer protection in subsequently challenged individuals. However, we concluded several years ago, prior to the performance of studies by ourselves and others which are summarized below, that the evidence in general supported the hypothesis that *protection against* influenza infection was probably due to the presence of neutralizing antibodies in the challenged hosts. We, therefore, have focused our main efforts on the analysis of the contribution of host factors towards the *recovery* process *from* influenza infection.

Recovery from Infection

In the past five years several laboratories have worked intensively in research areas related to the immune responses of the host to influenza infection and recovery from influenza infection following challenge. EFFROS *et al.* (12), ZWEERINK *et al.* (13), YAP *et al.* (14), ENNIS *et al.* (15), and BRACHIALE (16), have published reports which describe and characterize the cytotoxic-T lymphocyte response that follows the infection of mice with influenza viruses. The cells are detected by their ability to lyse chromium labelled target cells of various sorts. It became clear that two types of cytotoxic-T lymphocytes were induced, (a) subtype specific or (b) cross type reactive, i.e. capable of lysing cells bearing (a) any virus of the subtype or (b) viruses belonging to other subtypes. Neither of these cell types lysed cells bearing Type-B influenza virus antigens. The cross subtype reactive population of cytotoxic-T cells are of great interest because they appear to recognize determinants of a cross reactive antigen(s) which were not detected in serologic assays. This is the population of cytotoxic-T cells which is most readily detected following secondary antigenic stimulation with virus. It has been subsequently demonstrated [YAP *et al.* (17), ENNIS *et al.* (18)] that cytotoxic-T lymphocytes are also present in the lungs of mice infected with influenza pneumonia.

The transfer of influenza specific H-2 restricted cytotoxic-T lymphocytes protected recipient mice challenged with lethal influenza viruses [YAP *et al.* (19)]. WELLS *et al.* demonstrated that this transfer of protection was not associated with the detection of antibodies in recipients but by transfer of the cytotoxic-T

cells. In addition, transfer of antibody helping cells without cytotoxic-T cell activity conferred no protection to the lethally challenged mice when administered one to two days after challenge (20, 21). More recently, there have been reports of the establishment of cell lines which are derived from the subtype specific or cross reactive cytotoxic-T cell lines. LIN and ASKONAS have demonstrated that a murine cytotoxic-T cell line which has cytotoxic specificities restricted by H-2 antigens and is influenza specific protected recipient mice when transferred a murine cytotoxic-T cell line which has cytotoxic specificities restricted by H-2 antigens and is influenza specific protected recipient mice when transferred following challenge with virus (22). The protection was associated with a significant decrease in the pulmonary virus titer of recipient mice. In addition, this clone of cytotoxic-T cells produce gamma interferon when they recognized self and cross reactive virus antigen on target cells (23).

In other murine studies it was observed that inactivated whole virus vaccines induced cytotoxic-T cell responses in mice on primary immunization better than did vaccines made from disrupted virus (24, 25) but that mice given a large dose of inactivated virus, whether whole or subunit (26), or given a second dose of subunit vaccine following priming (25) also had an augmented cytotoxic-T cell response.

Studies in Humans

There is much less information available on the role of cytotoxic-T lymphocytes in humans during infection with influenza virus. However, valuable data have been collected in the short time since MCMICHAEL *et al.* (27) demonstrated HLA restriction of cell mediated lysis of influenza virus-infected human cells less than five years ago. SHAW and BIDDISON (28) also demonstrated an HLA genetic control of human cytotoxic-T cells following influenza infection with both influenza A and B viruses. In addition, both of these laboratories noted that some individuals with HLA A-2 antigen appeared to have a poorer cytotoxic-T cell response to influenza virus that did other individuals (29, 30). Following stimulation *in vitro* by influenza-infected stimulator cells, human peripheral blood leucocytes cultures which contain memory cells became cytotoxic when exposed to influenza infected cells *in vitro*. These cytotoxic assays employed as target cells peripheral blood leucocytes infected with influenza and labelled with chromium. The specificity of the killer cytotoxic cell was cross subtype reactive for influenza-A antigens. Most of the adult humans tested had memory cytotoxic-T cell activity which could be boosted following *in vitro* stimulation. MCMICHAEL *et al.* subsequently demonstrated that the activity of these *in vitro* stimulated lymphocytes was enhanced in five of eight volunteers who had received an inactivated whole virus vaccine, and in three of nine who had received subunit vaccine (31). In ongoing studies (personal communication) MCMICHAEL is evaluating the presence and level of memory cytotoxic-T cell activity prior to administration of live virus in normal volunteers in an attempt to correlate resistance to challenge with levels of serum antibody and memory cytotoxic-T cell activity and the clinical symptoms and virus shedding.

We have performed clinical investigations which were designed to detect HLA restricted virus specific cytotoxic-T cell responses following administration of live

virus, or inactivated vaccines in volunteers. Although it is most important to analyze these responses during natural influenza infection, it has not been logistically possible to obtain HLA typed lymphocytes from individuals before, during and after an epidemic. Therefore, we have obtained prospectively collected data using lymphocytes from HLA typed donors who have been given influenza virus as part of vaccine development studies. In the first study, five young adult volunteers were studied after innoculation with A/California (H1N1) virus. Three had systemic symptoms including fever and malaise as well as upper respiratory tract symptoms; they also shed virus in their nasal secretions. Four of the five had antibody responses detected using three techniques, and one individual had a rise in antibody detected only by the ELISA technique. The lymphocytes of four volunteers, three of whom has symptoms and shed virus, and another who had antibody responses detected by three techniques, were cytotoxic on HLA matched influenza virus infected target cells on days six or ten following infection, and were negative prior to infection and again by day 31. Thus, we were able to directly detect in the peripheral blood, the induction of HLA restricted virus specific cytotoxic-T cells during influenza infection (32).

In a study performed at the University of Sheffield, we studied the induction of HLA restricted virus specific cytotoxic-T cells in volunteers following administration of a live attenuated (H1N1) vaccine, a whole virus (H1N1) vaccine, or a purified vaccine containing surface antigens (HA and NA) of the H1N1 virus. There were 36 volunteers divided between the three groups. All three vaccines induced HLA restricted T-lymphocyte responses specific for influenza-A virus. This CTL response was found in 28 of 30 volunteers who developed antibody response and in three of six who did not develop antibodies. In this study we assessed-, memory cytotoxic-T cell activity following *in vitro* stimulation with virus-infected stimulator cells and also direct cytotoxicity using fresh peripheral blood leucocytes (CTL). A few volunteers had low levels of CTL response seven days after immunization, but the majority had detectable cytotoxic responses on day fourteen; although the mean level of lysis was low using fresh peripheral blood leucocytes as effector cells. When tested at day 0, the mean level of memory cytotoxic-T cell activity was about 5 percent and this increased more than two-fold by 7 days, and 6 to 7 fold by 14 days after immunization. By six months after vaccination, both the memory cytotoxic-T cell activity and the directly detected CTL activity had returned to preimmunization levels.

The CTL responses observed in this large study were overwhelmingly HLA restricted. Each of the 36 volunteers' lymphocytes were tested weekly for three weeks on five target cells chosen so that they would be HLA partially matched, or mismatched at HLA A and B loci. Of 83 instances where the effector lymphocytes shared an HLA A or B locus in common with the influenza virus infected target cells, 62 instances of influenza-A virus specific cytotoxicity were detected and in 21 instances no cytotoxic response was seen. On the contrary, using target cells which did not share in A or B locus in common with the effector cells, only eight influenza-A specific cytotoxic responses were observed and in 89 instances the cytotoxic results were negative (33).

Most of the volunteers produced both good antibody responses and an increase in cytotoxic-T cell activity. Of 13 individuals who received the inactivated whole

virus vaccine, 10 volunteers developed both antibody and specific CTL responses. Of the 3 remaining volunteers, 2 had obvious antibody responses without an increase in specific CTL activity and the third showed a positive HLA restricted virus specific CTL response without an antibody response. All 11 recipients of the surface antigen vaccine had both antibody and virus specific CTL responses after vaccination. The responses to the attenuated live virus vaccine were variable. Seven of the twelve volunteers who received the live vaccine developed both antibody and specific CTL responses, three volunteers showed neither an antibody nor a CTL response, and we assumed they were not infected by the attenuated virus. In the other two volunteers an HLA restricted virus-specific CTL response was detected in the absence of an increase in serum antibody (34).

When very high degrees of cytotoxic activity were observed on HLA matched target cells infected with the immunizing influenza H1N1 virus, after *in vitro* stimulation of lymphocytes obtained 14 days after vaccination, there was also some lysis of HLA mismatched target cells and of HLA matched target cells infected with the antigenically heterologous influenza-B virus. Thus after *in vitro* stimulation of lymphocytes obtained 14 days after vaccination, there was a pronounced increase in the ability of lymphocytes to kill HLA matched target cells infected with the virus used to stimulate the lymphocytes, and also a smaller increase in the ability of the *in vitro* restimulated cells to kill target cells which did not share an HLA antigen and those not specifically infected with the stimulating influenza-A virus. It is therefore likely that immunization induced a natural killer lymphocyte response in addition to the HLA restricted virus specific CTL response.

In a clinical study performed at the Common Cold Unit in Salisbury, we analyzed the natural killer lymphocyte activity of volunteers with influenza infection. It was known that infection with many viruses resulted in interferon production, and that interferon increases the activity of natural killer cells. We had earlier detected a rise in natural killer cell activity shortly after infection in a small group of volunteers infected with influenza A virus. The study in Salisbury was designed to confirm and extend those observations by assessing the level of natural killer cell activity in association with signs of illness, virus isolation, and interferon production during influenza infection in normal volunteers. Healthy adults received a live H1N1 virus intranasally. In the first study all eleven volunteers received virus and an increase in natural killer cell activity was detected in seven who shed virus on days 3 and 4, and in 3 of 4 individuals who did not shed virus. In the second study 17 volunteers were inoculated with virus, and 5 received placebo. There was a two- to three-fold increase in natural killer cell activity in samples collected three days after administration of virus, and this decreased by day six and returned to baseline by day 28, the next day tested. Interferon levels in the serum were increased on days three and six in individuals who had received virus; the levels of interferon correlated with the detection of virus shedding on days 3 and 4. Those shedding virus on both days had higher levels of serum interferon than those that shed on one day, or had no virus shedding. None of the recipients of placebo had detectable serum interferon, nor did they have an increase in natural killer cell activity (35).

The role of these natural killer lymphocytes in resistance against or in recovery

from virus infection, including influenza, is not clear. Nude mice have high levels of natural killer cell activity which are further elevated by influenza infection (DJEU and ENNIS *et al.*, unpublished data) but they do not clear virus from their lungs unless they are given virus specific H-2 restricted cytotoxic T cells (21, 22). Despite the apparent need for immunologically specific killer T cells to recover from influenza, early control of virus infection may be achieved by interferon induction which might protect host cells against infection, and also increase the activity of these natural killer lymphocytes. These earlier responses may help the host to control and limit virus infection until immunologically specific killer T cells are generated to destroy the virus infected cells, and T cells which aid in the production of antibody which is detected later during infection and appears to be important in resistance upon later challenge with virus.

Immune Interferon

Although this commentary deals with the roles of killer lymphocytes in influenza there is some interesting new information about the production of immune interferon by lymphocytes stimulated by influenza virus in conjunction with self antigens. During the Sheffield studies described above, we saved supernatent fluids from the lymphocyte cultures of the vaccinated individuals which had been restimulated *in vitro* with virus infected stimulated lymphocytes. We were surprised to find very high levels of interferon produced (10,000—50,000 IU/ml) and that 90 percent of the interferon was immune (gamma). Subsequently we studied the lymphocytes of normal blood donors and found they produced about 1,000 IU of interferon, about 60 percent of which appears to be immune (36). The influenza antigen responsible for inducing these high levels of gamma interferon is crossreactive among influenza A subtypes, including nonhuman influenza A viruses which are capable of stimulating human lymphocytes to produce interferons to a similar degree. Thus, it may be that the crossreactive antigen responsible for the generation of crossreactive influenza-A killer CTLs is also responsible for inducing immune interferon. The nature of the antigen responsible for the induction of gamma interferon has not been determined.

Recently MORRIS and ASKONAS reported that a clone of influenza-specific murine CTL produced gamma interferon *in vitro* when added to target cells which were infected with influenza A virus and also expressed H-2 antigens in common with those of the CTL line lymphocytes (23). Our observations were made using human lymphocytes some of which were infected with virus to act as stimulator cells while others from the same individual acted as responding cells. These two studies indicated that there are memory cells in the circulation which when exposed to virus-infected HLA- or H-2-matched stimulator cells, respond and produce high titres of immune interferon. Thus during influenza virus infections *in vivo*, specific memory lymphocytes may be stimulated by contact with influenza virus antigen which is presented in conjunction with HLA antigens and then aid host defences firstly by directly killing cells with virus antigen on their surfaces, and secondly by producing gamma interferon. The interferon could act directly by lowering the amount of infectious virus produced by cells in the respiratory

tract and indirectly by increasing the nonspecific killer cell activity which would aid in the removal of virus infected cells.

There are some reports of experiments on immunodeficient mice (nude or treated with cytotoxic drugs) in which animals survived rather longer than normal animals after receiving a lethal dose of virus. Thus, it is possible that T lymphocytes may have a detrimental as well as a beneficial role in the immune response to influenza virus. It is clear, however, that T lymphocytes are needed for the eventual clearance of virus from the lungs of the infected animal, in that mice with intact immune systems are better able to survive. It may be that early host responses involving such effector functions as natural killer cells, and interferon production help the host control infection with influenza until specific T lymphocyte mediated cytotoxicity develops.

Summary

In conclusion, we are beginning to have some understanding of the roles that the host immune responses may play in resisting influenza virus infection and in recovery of the host from influenza virus infection. Our present working hypothesis is that antibodies; particularly those in the respiratory tract, are probably most important in aiding the host resist challenge with aerosolized influenza virus. These antibodies might effectively neutralize some or all of the virus challenge and so reduce virus replication illness pathological changes. In the absence of adequate amounts of antibody, virus replication ensues in the respiratory epithelial cells. As a result of previous infection with cross-reactive strains, not recognized by antibody lymphocytes which have memory for influenza virus cross-reactive antigens may then initiate a secondary response of influenza-specific HLA-restricted killer lymphocytes and also produce both alpha and gamma (immune) interferon. The interferons would have antiviral activity by directly making cells resistant to virus infection, and indirectly by increasing the activity of natural killer cells which are present. These increases in interferon and natural killer cell activity precede the augmentation of memory cytotoxic T cell responses which occur in the peripheral blood of infected humans.

It also seems reasonable from the results of passive transfer studies in mice to conclude that once significant infection is under way and extensive virus replication has begun, cytotoxic T lymphocytes are needed to clear the virus infection and resolve the pneumonia (19—22). It does not appear that antibody is an effective means of clearing virus from the lung once infection is underway (21), but is probably important for resistance against later challenge.

Despite the advances in knowledge concerning the immune response of the host to influenza virus, there are still many aspects of the prevention and recovery from influenza infection that we do not understand. Although influenza infection of humans is the important problem which concerns us, we must continue to exploit animal models in order to obtain detailed information not available from human studies. Future investigations should include studies designed to detect the possible contribution of memory cytotoxic T cells in the respiratory tract towards resistance of the host to virus challenge. Studies on the memory cytotoxic T cells in mice following influenza infection have focused almost exclusively on

spleen cells as their source. In humans we are limited to the analysis of peripheral blood leucocytes in such studies. It would seem important to determine whether memory cytotoxic T cells can be detected in lymph nodes draining the respiratory tract and to compare the kinetics of these local responses with the responses of spleen cells on reinfection. We should also perform such experiments in mice following administration of inactivated vaccines, and determine whether cytotoxic T cells stimulated by inactivated vaccines are as protective when transferred to challenged mice, as those obtained from infected donor mice.

Future studies in vaccinated and infected humans should include detailed analyses of these cellular responses, as well as of systemic and local antibody responses using sensitive techniques. These studies should be done first in volunteers in order to correlate these indicators of host resistance with the results of challenge with infectious virus. It is most important that thereafter studies should be performed with these new assays in normal and vaccinated individuals before, during and after natural influenza virus challenge. These studies, logistically very difficult to perform will be necessary to provide better insights into the relationship between these assays and the *resistance* of humans *against* and *recovery from* influenza.

References

1. Louria, D. B., Blumenfeld, H. L., Ellis, J. T., Kilbourne, E. D., Rogers, D. E.: J. Clin. Invest. **38**, 213—265 (1959).
2. Doherty, P. C., Dunlop, M. B. C., Parish, C. R., Zinkernagel, R. M.: Inflammatory process in murine lymphocytic choriomeningitis is maximal in H-2 or H-2D compatible interactions. J. Immun. **117**, 187—190 (1976).
3. Welsh, R. M., Zinkernagel, R.: Heterospecific cytotoxic cell activity induced during the first three days of acute lymphocytic choriomeningitis virus infection in mice. Nature **268**, 646—664 (1977).
4. Fazekas de St. Groth, S., Donelly, M.: Studies in experimental immunology of influenza IV. Protective value of active immunization. Anst. J. Exp. Biol. Med. Sci. **28**, 61 (1950).
5. Schulman, J. L.: Effects of Immunity of Transmission of Influenza: Experimental Studies. Prog. Med. Virol. **12**, 128—160 (1970).
6. Virelizier, J. L.: Host defenses against influenza virus: The role of anti-haemagglutinin antibody. J. Immunol. **115**, 434—437 (1975).
7. Ennis, F. A., Wells, M. A., Barry, D. W., Daniel, S., Manischewitz, J.: Host defence mechanisms against influenza infection II Protection of mice with vacines against A/Port Chalmers/1/73 and B/Hong Kong/5/72. Postgrad. Med. J. **52**, 338 to 344 (1976).
8. Potter, C. W., Oxford, J. S., Shore, S. L., McLaren, C., Stuart-Harris, C.: Immunity to influenza in farrets. I. Response to live and killed virus. Brit. J. Exp. Path. **53**, 153—167 (1972).
9. Hobson, D., Curry, R. L., Beare, A. S., Ward-Gardner, A.: The role of serum hemagglutination-inhibitory antibody in protection against challenge infection with A2 and B viruses. J. Hyg. **70**, 767—777 (1972).
10. Couch, R. B., Kasel, J. A., Six, H. R., Cate, T. R.: The basis for immunity to influenza in man. In: Nayak, D. P., Fox, C. F. (eds.), Genetic Variation among Influenza Viruses. ICN-UCLA Symposia on Molecular and Cellular Biology XXI, 535—546 (1981).
11. Murphy, B. R., Phelan, M. A., Nelson, D. L., Yarchan, R., Tuoney, E. L., Alling, D. W., Tierney, E. L.: Hemagglutinin-specific enzyme linked immunosorbent assay for antibodies to influenza A and B viruses. J. Clin. Micro. **13**, 554 to 560 (1981).

12. Effros, R. B., Doherty, P. C., Gerhard, W. E., Bennink, J.: Generation of both cross-reactive and virus specific cytotoxic T cell population after immunization with serologically distinct influenza A viruses. J. Exp. Med. **145**, 557—558 (1977).
13. Zweerink, H. J., Courtneidge, S. A., Skehel, J. J., Crumpton, M. J., Askonas, B. A.: Cytotoxic T cells kill influenza virus infected cells but do not distinguish between serologically distinct influenza A virus. Nature **267**, 354—356 (1977).
14. Yap, K. L., Ada, G. L.: Cytotoxic T cells specific for influenza virus infected target cells. Immunology **32**, 151—159 (1977).
15. Ennis, F. A., Martin, W. J., Verbonitz, M. W.: Haemagglutinin specific cytotoxic T cell responses during influenza infection. J. Exp. Med. **146**, 843—898 (1977).
16. Brachiale, T. J.: Immunologic recognition of influenza infected cells: 1. Generation of virus stain specific and cross-reactive subpopulations of cytotoxic T cells in response to type A influenza virus infection of different subtypes. Cell Immunol. **33**, 423—426 (1977).
17. Ennis, F. A., Wells, M. A., Butchko, G. M., Albrecht, P.: Evidence that cytotoxic T cells are part of the host's response to influenza pneumonia. J. Exp. Med. **148**, 1241—1249 (1978).
18. Yap, K. L., Ada, G. L.: Cytotoxic T cells in the lungs of mice infected with an Influenza A virus. Scand. J. Immunol. **7**, 73—80 (1978).
19. Yap, K. L., Ada, G. L., McKenzie, I. F. C.: Transfer of specific cytotoxic T lymphocytes protects mice inoculated with influenza viruses. Nature **273**, 238—239 (1978).
20. Wells, M. A., Albrecht, P., Ennis, F. A.: Recovery from a viral respiratory tract infection. I. Influenza pneumonia in normal and T deficient mice. J. Immunol. **126**, 1036—1041 (1981).
21. Wells, M. A., Ennis, F. A., Albrecht, P.: Recovery from a viral respiratory tract infection II. Passive transfer of immune spleen cells to mice with influenza pneumonia. J. Immunol. **126**, 1042—1046 (1981).
22. Lin, Y.-L., Askonas, B. A.: Biological properties of an influenza virus killer T cell clone. J. Exp. Med. **154**, 225—234 (1981).
23. Morris, A. G., Lin, Y.-L., Askonas, B. A.: Immune interferon release when a cloned cytotoxic T cell line meets its correct influenza infected target cell. Nature **295**, 150—152 (1982).
24. Webster, R. G., Askonas, B. A.: Cross-protective and cross-reactive cytotoxic T cells induced by influenza virus vaccine in mice. Eur. J. Immunol. **10**, 396—401 (1980).
25. Reiss, C. S., Schulman, J. L.: Cellular immune responses of mice to influenza virus vaccines. J. Immunol. **125**, 2182—2188 (1980).
26. Ennis, F. A., Martin, W. J., Verbonitz, M. W.: Cytotoxic T lymphocytes induced in mice by inactivated influenza vaccine. Nature **266**, 418—419 (1977).
27. McMichael, A. J., Ting, A., Zweerink, H. J., Askonas, B. A.: HLA restriction of cell mediated lysis of influenza virus infected human cells. Nature **270**, 524—526 (1977).
28. Shaw, S., Biddison, W. E.: HLA-linked genetic control of the specificity of human cytotoxic T cell responses to influenza virus. J. Exp. Med. **149**, 565—575 (1979).
29. McMichael, A. J.: HLA restriction of human cytotoxic T lymphocytes specific for influenza virus. Poor recognition of virus associated with HLA A2. J. Exp. Med. **148**, 1458—1467 (1978).
30. Biddison, W. E., Payne, S. M., Shearer, G. M., Shaw, S.: Human cytotoxic T cell responses to trinatrophenyl happen and influenza viruses. J. Exp. Med. **152**, 204—217 (1980).
31. McMichael, A. J., Gotch, F., Cullen, P., Askonas, B. A., Webster, R. G.: The human cytotoxic T cell response to influenza vaccination. Clin. Exp. Immunol. **43**, 276—284 (1981).

32. Daisy, J. A., Tolpin, M. D., Quinnan, G. V., Rook, A. H., Murphy, B. R., Mittal, K., Clemenis, M. L., Mullinix, M. G., Kiley, S. C., Ennis, F. A.: Cytotoxic cellular immune responses during Influenza A infection in human volunteers. In: Bishop, D. M. L., Compams, R. W. (eds.), The replication of negative strand virus, 443—448. Elsevier/North-Holland 1981.
33. Ennis, F. A., Rook, A. H., Yi-Hua, Q., Schild, G. C., Riley, D., Pratt, R., Potter, C. W.: HLA restricted virus specific cytotoxic T lymphocyte responses responses to live and inactivated influenza viruses. to live and inactivated influenza viruses. Lancet 887—891 (1981).
34. Ennis, F. A., Yi-Hua, Q., Schild, G. C.: Antibody and cytotoxic T lymphocyte responses of humans to live and inactivated influenza vaccines. J. gen. Virol. (1982).
35. Ennis, F. A., Meager, A., Beane, A. S., Yi-Hua, Q., Riley, D., Schwary, G., Schild, G. C., Rook, A. H.: Interferon induction and increased natural-killer cell activity in influenza infections in man. Lancet 891—893 (1981).
36. Ennis, F. A., Meager, A.: Immune interferon produced to high levels of antigenic stimulation of human lymphocytes with influenza virus. J. Exp. Med. *1981*, 1279—1289.

Author's address: Dr. F. A. Ennis, Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01605, U.S.A.

Received June 3, 1982