Severity of Fever in Influenza: Studies on the Relation between Viral Surface Antigens, Pyrexia, Level of Nasal Virus and Inflammatory Response in the Ferret

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(Accepted 15 March 1985)

SUMMARY

Previous work has shown that fever in influenza of ferrets occurs following release of endogenous pyrogen from virus–phagocyte interaction in the upper respiratory tract (URT), and suggested that the poor inflammatory response and correspondingly low fever elicited by A/Puerto Rico/8/34 (H1N1), compared with H3N2 reassortant clones of A/Puerto Rico/8/34–A/England/939/69, were related to its H1 and N1 surface antigens. Nasal virus levels, inflammatory and pyrexial responses produced in ferrets by clones 31 (H3N1) and 64b (H1N2) of the same reassortant system suggested a connection between the H1 antigen and low inflammatory response, but results were not conclusive. Unlike A/Puerto Rico/8/34, two recent H1N1 isolates, A/USSR/90/77 and A/Fiji/15899/83, produced a high inflammatory response yet low fever despite large amounts of virus in the URT, suggesting that either no connection exists between the acquisition of the H1 antigen and production of a low inflammatory response, or the H1 antigen of recent isolates, whilst antigenically related to that of A/Puerto Rico/8/34, is biologically different.

The constitutional effects of influenza are fever, headache, myalgia, listlessness, nausea, shivering, anorexia and depression (Fenner et al., 1974). Influenza virus produces fever, as do many other agents, by stimulating the release of endogenous pyrogen (EP) from mononuclear phagocytes (Dinarello & Wolff, 1982; Gander, 1982). Inoculation of human volunteers with human EP has been shown to produce, in addition to fever, the other constitutional effects of influenza (Rawlins & Cranston, 1973). Our studies on human influenza in the ferret model (Sweet et al., 1979) indicated that fever resulted from production of EP from phagocytes following interaction with virus in the upper respiratory tract (URT), suggesting that all the constitutional effects of influenza could follow from infection at this locus. Furthermore, with regard to differences in the severity of constitutional effects produced by different influenza virus strains in humans, strains of the A/Puerto Rico/8/34–A/England/939/69 (H3N2) reassortant virus system showed different capacities to produce fever in ferrets (Toms et al., 1977; Matsuyama et al., 1980). The parent strain, A/Puerto Rico/8/34 (PR/8) (H1N1), and reassortant clone 64d (H3N2) produced low pyrexial responses during infection, whereas clones 7a (H3N2) and 64c (H3N2) produced high febrile temperatures. These differences in pyrexial response could be due to (i) varying quantities of virus particles available for interaction with phagocytes in the URT, (ii) varying numbers of phagocytes in the URT, (iii) differences between strains in their ability to stimulate EP release from phagocytes, or (iv) a combination of the above factors. In our ferret studies, the infections produced by the H3N2 clones 64d, 7a and 64c simulated similar numbers of phagocytes in the nasal inflammatory response (Toms et al., 1977; Matsuyama et al., 1980) and the low fever produced by clone 64d was probably due to the

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smaller amounts of virus present in the URT compared with the other two clones. The high febrile response produced by clone 64c, in spite of its lower growth in the URT compared with clone 7a, may indicate that it possesses a greater capacity to induce EP (Matsuyama et al., 1980).

The work presented in this paper relates to the infection produced by the H1N1 strain PR/8, which is characterized by a low febrile response despite high levels of virus growth in the URT (Matsuyama et al., 1980). This low febrile response may be related to the lower and somewhat delayed (compared with the H3N2 clones 64d, 7a and 64c) URT inflammatory response [see (ii) above] produced by PR/8 infections. In order to determine whether the poor inflammatory response to PR/8 is related specifically to the H1 and/or N1 surface antigens, virus levels and inflammatory and pyrexial responses were monitored in ferrets inoculated with two further clones, 31 (H3N1) and 64b (H1N2), of the A/Puerto Rico/8/34–A/England/939/69 reassortant virus system. Both clones derived at least five of the six genes coding for non-surface antigen polypeptides from PR/8, the origin of the sixth gene being unknown (Oxford et al., 1978). To ascertain whether a poor inflammatory response and low fever is general for H1N1 viruses, two recent H1N1 isolates [A/USSR/90/77 (A/USSR) and A/Fiji/15899/83 (A/Fiji)] were examined in ferrets.

Clones 31 (H3N1) and 64b (H1N2) were supplied by Dr A. S. Beare (Common Cold Research Centre, Salisbury, U.K.), and wild-type viruses, A/USSR (H1N1) and A/Fiji (H1N1), by Dr J. J. Skehel (World Influenza Centre, National Institute for Medical Research, Mill Hill, London, U.K.). Seed and working stocks were prepared as described previously for other strains (Sweet et al., 1974). Their assay in eggs and allantois-on-shell cultures (egg-bits) were also as described previously (Sweet et al., 1974), except that egg-bits were incubated for 72 h rather than 48 h as this increased the sensitivity of the assay for the wild-type viruses. Virus in nasal washings was titrated in egg-bits (EBID50 = 50% egg-bit infectious dose), but to allow comparison of titres in terms of the more sensitive but less convenient egg assay (EID50 = 50% egg infectious dose), EID50/EBID50 ratios for the four strains were determined (log10 EID50/ml of virus — log10 EBID50/ml of virus); these were 0.7, 1.0, 1.1 and 1.2 for clone 31, clone 64b, A/USSR and A/Fiji, respectively.

Adult male ferrets, obtained from A. S. Roe (Little Fakenham, Norfolk, U.K.) were inoculated intranasally, under ether anaesthesia, with 10^7 EID50 of virus, as described by Toms et al. (1976, 1977), and nasal washings obtained at 6 to 24 hourly intervals up to 96 h post-inoculation. Collection of nasal washings and counts of total inflammatory cells in these washings have been described (Toms et al., 1976, 1977). Following inoculation, rectal temperatures were measured using a digital thermometer (R. S. Components, Birmingham, U.K.), with an accuracy of 0.1 °C over the range -50.0 °C to +199.9 °C, attached to a type K thermocouple input. Fever was considered significant if the rectal temperature rose by >0.7 °C above the mean pre-infection temperature (determined as described by Toms et al., 1977), a rise of 0.7 °C being twice the standard deviation of the average pre-infection mean (Campbell et al., 1979). The rise was plotted against time post-inoculation and the level of fever was assessed by determining the area under the curve >0.7 °C, as described by Campbell et al. (1979).

Ferrets inoculated with clone 31 (H3N1) (Fig. 1a) produced a high inflammatory response which reached a maximum (10^6-9 cells) at 36 h and remained high up to 96 h post-infection. Virus titres in the URT peaked earlier, at 24 h post-infection, and were also relatively high: 10^5.4 EBID50/ml of nasal wash, equivalent to 10^6.4 EID50/ml. The febrile response, which lasted approximately 24 h, was also high, with a peak mean rise in rectal temperature of 2.2 °C between 30 and 36 h post-infection; the fever level, measured in arbitrary units from the areas under the curves for individual animals, was 26. In contrast, infection with clone 64b (H1N2) (Fig. 1b) produced a low inflammatory response (2- to 13-fold lower cell numbers in nasal washes compared with clone 31) and a lower and shorter fever (peak of 1.8 °C at 36 h post-infection: fever level of 22 arbitrary units). This situation was similar to that found for PR/8 (Matsuyama et al., 1980); however, unlike PR/8, nasal virus titres were also low, peaking at 17 h post-infection, with a mean of 10^6.5 EBID50/ml, equivalent to 10^6.5 EID50/ml.

Both recent H1N1 isolates grew well in the URT of infected ferrets (Fig. 2a, b), producing mean titres of 10^5.1 EBID50/ml (equivalent to 10^6.2 EID50/ml) at 30 h post-infection for
Fig. 1. Mean titres of virus (log_{10} EBID_{50}/ml) in nasal washes (■), mean total inflammatory cell counts (log_{10}) in nasal washes (▲) and mean changes in rectal temperature (°C) (●) of ferrets intranasally inoculated with (a) clone 31 (H3N1) and (b) clone 64b (H1N2) of the A/Puerto Rico/8/34–A/England/939/69 recombinant virus system. Means of virus titres and cell counts are geometric, and means of temperature changes are arithmetic; they are for six animals in (a) and five animals in (b). Bars represent the standard error of the mean.

A/USSR, and 10^{5.3} EBID_{50}/ml (equivalent to 10^{6.5} EID_{50}/ml) at 24 h post-infection for A/Fiji. Unlike PR/8 (H1N1) and clone 64b (H1N2), however, both viruses elicited high inflammatory responses (means of 10^{7.0} cells for A/USSR and 10^{7.1} cells for A/Fiji, at 42 h post-infection), yet pyrexial temperatures for both infections were low (maximum mean of +1.7 °C for A/USSR and +1.3 °C for A/Fiji, and fever levels of 10 and 6 arbitrary units, respectively).

These studies have confirmed two previous observations. Firstly, the onset and rise of fever induced by clones 64b and 31 and the recent H1N1 isolates coincided with the onset and rise of the inflammatory response (Fig. 1 and 2), further suggesting a link between these two parameters. Secondly, the decline in virus titres in the URT correlated with the onset of the inflammatory response and pyrexia (Fig. 1 and 2), again suggesting a role for these host responses in limiting URT infection.

The results for clone 31 (H3N1) of the A/Puerto Rico/8/34–A/England/939/69 reassortant virus system supported the possibility that the poor inflammatory response to the H1N1 parent virus PR/8 might be due to the H1 antigen. This clone, which derived only the gene coding for
Fig. 2. Mean titres of virus (log_{10} EBID_{50}/ml) in nasal washes (■), mean total inflammatory cell counts (log_{10}) in nasal washes (▲), and mean changes in rectal temperature (°C) (●) of ferrets intranasally inoculated with (a) A/USSR/90/77 (H1N1) and (b) A/Fiji/15899/83 (H1N1). Means of virus titres and cell counts are geometric and means of temperature changes are arithmetic; they are for 20 animals in (a) and six animals in (b). Bars represent the standard error of the mean.

The results obtained for A/USSR and A/Fiji show that natural H1N1 isolates can replicate well in the URT of ferrets and stimulate a high inflammatory response (Fig. 2). Clearly, therefore, not all viruses bearing H1N1 surface glycoproteins stimulate a poor inflammatory response, suggesting either that the H1 protein is not involved in eliciting this response or that

the haemagglutinin from its H3N2 parent (Oxford et al., 1978), replicated as well as PR/8 in the URT, but unlike PR/8 elicited a high inflammatory response and a correspondingly high fever (Fig. 1 a). Furthermore, clone 64b (H1N2) whose genetic composition is essentially the same as PR/8 except for its neuraminidase (Oxford et al., 1978) stimulated a poor inflammatory response and a low fever (Fig. 1 b). Unfortunately, however, unlike PR/8, clone 64b did not grow well in the URT and thus the low inflammatory response and the low fever may have been due to poor production of virus rather than its H1 antigen. In the absence of a H1N2 reassortant which grows well in the URT yet elicits a low inflammatory response and fever, the question of relating inflammatory response and pyrexia to particular surface antigens cannot be answered conclusively.
the haemagglutinins of the modern H1N1 viruses, whilst antigenically related to that of PR/8, have different biological properties. Since its isolation, PR/8 has been passaged many times in vitro and in experimental animals and it is not surprising, therefore, that such stocks of PR/8 exhibit characteristics different to those of old or recent natural H1N1 isolates. However, it was surprising that the modern H1N1 isolates elicited relatively high levels of both virus and phagocytes but a low fever. The possibility that A/USSR and A/Fiji are less able to stimulate EP release from phagocytes, compared with clones 31, 7a and 64c, is being investigated.

This work was supported by a project grant from the Wellcome Trust.

REFERENCES


(Received 4 February 1985)