Evaluation of immunity to Aujeszky's disease virus

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Summary: The authors studied the immune response of vaccinated and unvaccinated pigs to challenge infection with Aujeszky's disease virus. One group received a live vaccine by the nasal route, a second group was inoculated intramuscularly with inactivated vaccine, and a third group served as unvaccinated controls. General and local immune responses were measured by indirect ELISA, serum neutralisation, antibody-dependent cellular cytotoxicity assay, and complement-dependent antibody lysis. Weight gain was chosen as the quantitative criterion for the clinical consequences of experimental infection. A discussion of the results considers the limitations of in vitro tests as reflections of the degree of immune protection occurring in vivo. In general, nasal vaccination conferred a better immunity to experimental infection than parenteral vaccination.


INTRODUCTION

Vaccines against herpes viruses which give good protection against infection and disease have proved difficult to prepare. Such is the situation with Aujeszky's disease virus (ADV), where vaccines may only give poor protection against disease, often require two inoculations, are severely hampered by maternal antibody and often only produce short lived immunity. Despite this, the literature contains little precise information on what constitutes an ADV protective response, although empirically based experiments with ADV have led to two conclusions: firstly, that serum antibody titres give little guide to the efficacy of ADV vaccines (6) and secondly, that intranasal versus intramuscular vaccination gives superior protection (2). With these facts in mind we have studied the systemic and local immune response in animals vaccinated with commercial vaccines and tried to correlate our findings with the ability of these animals to withstand challenge from a field isolate.

SYSTEMIC ANTIBODY RESPONSES AGAINST AUJESZKY'S DISEASE VIRUS

Vaccines and viruses

One group was intramuscularly inoculated with an inactivated virus vaccine (Pseudorabies vaccine, Salsbury Laboratories, USA) and challenged 43 days later. The second group was given two intramuscular inoculations, with a 3-week interval, using a live attenuated vaccine (Duvaxyn Aujeszky, Duphar, Holland). Animals were challenged 13 days after the second inoculation. The third group acted as
an unvaccinated control group and was similarly challenged by intranasal inoculation (1 ml/nostril) of the Danish isolate U298/81 at $5 \times 10^6$ pfu/ml.

**Sampling and clinical examination**

Serum samples were taken from animals prior to vaccination and challenge and during the course of clinical disease.

After separation and heat inactivation (56°C, 30 minutes), serum was stored at −20°C before use. Animals were examined daily for clinical signs and weighed before and after challenge.

**Assays**

Serum was used to measure anti-Aujeszky’s disease virus activity in an indirect ELISA, details of which have been published previously (5). Virus neutralising activity of serum was measured by incubating various dilutions of serum with a constant amount of virus at 37°C for 24 hours, according to the method of Bitsch and Eskildsen (1). Antibody dependent cellular cytotoxicity (ADCC) and complement dependent antibody lysis (CDAL) were measured in assays, the details of which have been published previously (5).

**Data analysis**

Sera taken from pigs on the day before challenge were titrated and the area under the titration curve for each sample taken as a measurement of the quantity of antibody detected by each test. A similar plot of weight gains after the day of challenge was also computed for each pig and regression analysis of these two sets of data was used to indicate any correlation between antibody levels and protection against disease.

**RESULTS AND DISCUSSION**

The duration and intensity of the clinical signs in the vaccinated groups were reduced compared to the controls, although neither vaccine gave complete protection against either infection or disease.

After vaccination, each of the serological tests could detect specific antibody against ADV. Despite a second inoculation of live virus vaccine after 20 days, there appeared to be little difference between the titres of the two vaccine groups at the time of challenge. After intranasal challenge, antibody titres in vaccinated animals rose in an anamnestic fashion, whereas titres in control animals rose more slowly and reached lower levels. By 20 days post-challenge (DPC), complement dependent antibody levels were significantly higher in the live vaccine group, whereas ADCC and neutralisation titres were similar for both groups.

On the day prior to challenge, serum from the vaccinated groups was titrated and tested in the complement, ADCC and neutralisation assays and compared to the weight gains which pigs showed after challenge. There was no correlation between the quantity of antibody, as measured by these tests, and protection against clinical disease, as measured by weight gains. This is illustrated in Figs. 1 and 2, which show weight gains of some animals from the live and inactivated vaccine groups and titrations of complement and ADCC antibody. Thus, in Fig.1, in the
live vaccine group the first two pigs show similar weight gain patterns, with one pig showing a reasonable ADCC titre and the other showing no detectable activity. In the inactivated group (Fig. 2), data from the first and last pigs illustrated show similar complement dependent antibody lysis although the weight gains are entirely different.

The results extend the serological observations on neutralising antibody (6) by also showing that neither complement dependent antibody lysis nor ADCC titres appear to correlate with protection.

There are three possible explanations for this result. Firstly, weight gains may not be a suitable method for measuring the severity of disease. Secondly, our in vitro assays, as measures of functional immunity, may not correspond to the way in which they act in vivo. Thirdly, there may not be any correlation between these effector mechanisms and protection.

Quantitative measurement of the severity of disease is difficult and, because of the added difficulty of handling and clinically examining pigs infected with ADV, an even greater problem exists. Previous investigators have used pyrexia, viral excretion and weight gains to assess the severity of disease after challenge (6, 10) and weight gains have become an acceptable quantitative indicator of the protection which ADV vaccines afford.

The second consideration is even more complex and centres on how closely the technological compromises necessary to perform in vitro assays make them only a poor reflection of the situation in vivo. These compromises concern both the conditions necessary to perform each individual test and the fact that other possible conflicting mechanisms, which are obviously present in vivo, are excluded from in vitro tests, to allow more simple measurements to be made. However, because of the stability of antibody and the fact that it does not fluctuate as markedly as other soluble factors which could influence the action of cells involved in the destruction of virus-infected cells, humoral measurement of immunity is normally considered to give more reproducible values than measurement of cellular effects. To assess in vivo importance, antibody can be transferred to susceptible animals prior to challenge, but this can be confusing because of the many different ways in which antibody can act. In small animal models, this may be partly overcome by depleting animals of a particular component and, hence, studying one factor in isolation. However, in large animals this is more difficult and there has therefore been, in the past, more reliance on the straight association between antibody levels and protection, despite the reservation of the possible plurality of that antibody response. More recently, however, reports of isolated ADCC systems have appeared (4) and, in another disease of pigs, African swine fever, which does not produce neutralising antibody, it has been possible to assess the importance of both complement and ADCC mechanisms to protection in vivo and to show that in vitro measurements appear to correlate well with resistance to disease (11). This would argue that in a similar pig system with ADV our in vitro measurements would be expected to have relevance to the functionally important mechanisms in vivo.

Such conclusions only leave the consideration that our systemic measurements of immunity have little value in assessing whether animals are likely to be protected from ADV challenge. Viral replication, in pigs infected with ADV by the nasal route, takes place predominantly in tissues of the head and neck. Systemic spread is rare and probably occurs only when infected cells escape into the blood stream.
RECIPROCAL ANTIBODY DILUTION

FIG. 1

Weight gain (●—●) after challenge and ADCC antibody (★★) at time of challenge.
Panel A live vaccine group; Panel B inactivated vaccine group.
DPI: days post infection; %SL: percentage specific lysis of infected cells in ADCC assay.
FIG. 2

Weight gain (•—•) after challenge and CDAL antibody (*---*) at time of challenge.

Panel A live vaccine group; Panel B inactivated vaccine group.

DPI: days post infection; %SL: percentage specific lysis of infected cells in CDAL assay.
After multiplication in the nasal and tonsillar mucosae, there is a rapid spread to the olfactory bulb and studies with ADV in a number of species have shown the importance of this neural spread. Once within nerves, virus is inaccessible to many immunological mechanisms; hence, the virus must be controlled locally before it enters the nerve (3) or centrally once it gains access to the brain's stem. Ligation and nerve section experiments in mice with ADV suggested that control at a local level could occur (3) and it is therefore immunological factors at this level which might provide the best assessment of the animal's resistance to challenge. Indeed, intranasal inoculation of ADV vaccines has shown marked success (2) and work with herpes simplex virus in mice has shown the importance of locally active immune mechanisms (8).

These arguments suggest that far too much emphasis has been placed on measurement of systemic immunity and that, to understand the host's response to ADV, functional analysis of local immunological events will be necessary.

LOCAL HUMORAL AND CELLULAR RESPONSES AGAINST AUJESZKY'S DISEASE VIRUS

Vaccines and viruses

Similar groups of animals were used as described above, except that a fourth group was included which received a live attenuated vaccine intranasally and was challenged 28 days later.

Sampling and assays

ADCC, CDAL, ELISA and serum neutralisation assays were carried out as described above. Direct lymphocyte cytotoxicity of lymph node suspensions in pigs given intranasal vaccine was measured according to the method of Norley and Wardley (9). Tracheal and nasal washings were collected according to the method of Morgan and Bourne (7).

RESULTS AND DISCUSSION

In contrast to parenterally vaccinated groups, the intranasally vaccinated group did not lose weight and continued to fatten. As a group, this set of animals showed a much reduced temperature response although all the vaccinated animals excreted virus for a similar period after challenge.

The Aujeszky's specific antibody activity in the tracheal and nasal washings from the 3 vaccine groups at the time of challenge was characterised by the following features: 1) The most consistent finding was an IgA and ADCC response. 2) No neutralising antibody was detected and CDAL was found in only one sample. 3) IgG was present mainly in the secretions from the trachea, but only in very low amounts.

A somewhat surprising finding, however, was that animals given live virus intramuscularly produced a better IgA and ADCC response than animals vaccinated intranasally. Animals given inactivated vaccines produced a good ADCC response with only trace amounts of IgA.
However, neither CDAL or ADCC mechanisms correlated with protection and our inability to show good neutralising activity in nasal and tracheal washings again reflects the situation systemically where serum neutralising titres do not correlate with protection. These results indicate that, as with other herpes viruses, protection may be more closely allied to cellular responses and reside at the level of the cytotoxic and helper T cell.

Experiments on the cytotoxic activity of cells from local lymph nodes showed that, by day 2 post-challenge, low levels of cytotoxicity were evident against all the targets, indicating that the killing was not host restricted. By day 6 one pig showed high levels of cytotoxicity with an obvious preference for ADV-infected autologous fibroblasts. By day 10 the level of cytotoxicity had decreased, but again it appeared host restricted. These results can be interpreted as indicating that ADV infection stimulates a short-lived cytotoxic T cell response in the draining lymph node of infected pigs.

Work with other viruses which cause respiratory disease have shown the importance of specific mucosal immunity and that local application of vaccine is considered to give better stimulation of local antibody. This did not appear to be the case with our ADV experiments and, despite the resistance of our intranasally vaccinated groups to challenge, there appeared to be no correlation between our local humoral measurements and protection.

**CONCLUSION**

The fact that the correlation between local mucosal antibodies and protection was poor is perhaps not surprising when one considers that, although vaccinated pigs are protected against lethal challenge, they are unable to prevent virus from reaching the brain. The extent of these brain lesions is much reduced compared to control animals, suggesting that factors in addition to mucosal immunity are also active in vaccinated animals. Such mechanisms would presumably be localised in the lymph nodes draining the site of virus replication and the direct lymphocyte cytotoxicity which we were able to measure in the lymph nodes draining the tonsillar region of infected pigs may be an important antiviral effector mechanism. Thus, as neither humoral mucosal factors nor systemic antibodies appear to correlate to protection, despite its cost and technical difficulty, anti-ADV cellular activity in the draining lymph nodes of infected animals deserves close scrutiny.

**ÉVALUATION DE L'IMMUNITÉ GÉNÉRALE ET LOCALE CONTRE LE VIRUS DE LA MALADIE D'AUJESZKY. — S. Martin et R.C. Wardley.**

Résumé : Les auteurs étudient les réponses immunitaires des porcs vaccinés ou non, soumis à une infection d'épreuve par le virus de la maladie d'Aujeszky. Deux lots sont vaccinés soit avec un vaccin vivant administré par voie intranasale, soit avec un vaccin inactivé par voie intramusculaire, un troisième lot non vacciné étant maintenu comme témoin. Les réponses immunitaires générales et locales sont mesurées par les méthodes ELISA indirecte, de séroneutralisation, de cytotoxicité cellulaire anticorps-dépendante et de lyse d'anticorps complément-dépendante. Pour prendre en compte les conséquences cliniques...
de l'infection expérimentale, le gain de poids a été choisi comme critère quantitatif. Les résultats sont interprétés et discutés en considérant les limites des épreuves in vitro par rapport à la situation réelle in vivo pour évaluer le degré de protection immunitaire. Globalement, la vaccination intranasale a conféré une meilleure protection contre l'infection expérimentale que la vaccination parentérale.


EVALUACIÓN DE LA INMUNIDAD GENERAL Y LOCAL CONTRA EL VIRUS DE LA ENFERMEDAD DE AUJESZKY. — S. Martin y R.C. Wardley.

Resumen : Estudian los autores las respuestas inmunitarias de los cerdos vacunados o no, sometidos a una infección de prueba por el virus de la enfermedad de Aujeszky. Se vacunan dos lotes, bien sea con una vacuna viva administrada por vía intranasal, o bien con una vacuna inactivada por vía intramuscular, manteniéndose un tercer lote sin vacunar como testigo. Se miden las respuestas inmunitarias generales y locales con los métodos ELISA indirecto, seroneutralización, citotoxicidad celular anticuerpo-dependiente y lisis de anticuerpo complemento-dependiente. Para tener en cuenta las consecuencias clínicas de la infección experimental, se adoptó la ganancia de peso como criterio cuantitativo. Se interpretan los resultados y se los discute considerando los límites de las pruebas in vitro con relación a la situación real in vivo para evaluar el grado de protección inmunitaria. Globalmente, la vacunación intranasal confirió mejor protección contra la infección experimental que la vacunación parenteral.


REFERENCES


