Marek's Disease in Chickens: Development of Viral Antigen in Feather Follicles and of Circulating Antibodies

F. STECK* and H. U. HABERSTICH

Institute of Microbiology, School of Veterinary Medicine, University of Berne, Switzerland

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The infection of young chickens with Marek's disease herpesvirus (MDHV) leads to the early appearance of viral antigen in the bursa Fabricii, in the kidney tubular epithelium, and particularly in the epithelial cells of growing feather follicles (Calnek and Hitchner, 1969; Purchase, 1970). Viral antigen may persist in feather follicles over several months, whereas in neural lesions or in lymphoid tumors induced by MDHV viral antigen is either lacking or present only in a few cells (Calnek and Hitchner, 1969; Spencer and Calnek, 1970; Purchase, 1970). Haider et al. (1976) have pointed out the high diagnostic value of the double-diffusion agar-gel precipitation test (Chubb and Churchill, 1968) using growing feathers of MDHV-infected chickens as antigen.

In the following experiments we studied the appearance and persistence of feather follicle (FF) antigen after infection, as detected by the gel precipitation test, in unvaccinated chickens and in chickens vaccinated with the herpesvirus of turkeys (HVT). The presence of antigen was compared with the formation of precipitating and neutralizing antibodies and with the development of clinical disease.

MATERIALS AND METHODS

Chickens. A local commercial breed of white Leghorn hybrid (Schweiz. Geflügelzuchtschule, Zollikofen) was used in all experiments.

Infection experiments. A longitudinal study of the development viral antigen and circulating antibody was conducted in vaccinated and unvaccinated chickens after infection with virulent Marek's disease virus (MDHV). The experiment is described under Results (iii).

For comparison, eight experimental groups of birds, all of the same breed and infected with the same strain of MDHV at the age of 1 to 20 days and killed after various time intervals up to the age of about 16 weeks, were evaluated together (Fig. 4; Tables 3 and 4).

Isolators. Groups of up to five chickens were held in isolators under slight positive pressure with filtered ingoing and outgoing air, thermoregulation, and automatic water supply. A large food container allowed feeding during the first 3 weeks without refilling. Refilling was done from the outside without interruption of the isolation. Fecal matter was collected in a bin with a 3-month holding capacity placed under a wire-mesh tray covering the whole floor.

Virus strains. A virulent strain of MDHV was isolated by direct kidney culture (23) from a white Leghorn chicken with the classical form of Marek's disease (MD): neuritis of plexus ischiadicus with moderate lymphoreticular infiltration. This virulent strain was used in the first or second passage in cultures of embryonic chicken kidney cells or, in some instances, after one additional passage in chickens and reisolation by direct kidney cultures. Infections were done by the intra-abdominal (i.a.) inoculation of infected culture cells. The number of cells inoculated per bird corresponded to 10 to 100 plaques in the original cell culture.

The same virus isolated was passaged by cell transfer in embryonic chicken kidney cultures. It was avirulent for 1-day-old chicks after passage 32 in culture (MDHV-TC) (unpublished data).

MDHV-infected cell suspensions at low or high tissue culture passage level were preserved frozen at −196 C in liquid nitrogen after addition of 10% dimethyl sulfoxide (20).

Vaccine virus. HVT (live vaccine of the TAD Co., Cuxhaven) was passaged by cell transfer six times in chicken embryo cell cultures, liberated by ultrasonic treatment, and stored as a cell-free suspension at −70 C. For immunization, chickens were inoculated i.a. with about 2,000 plaque-forming units of HVT.

Cell cultures. Direct kidney cultures for virus isolation from infected birds were done according to the method described by Witter et al. (23).

Chicken embryo cell cultures of 10-day-old embryos and embryonic chicken kidney cultures of 18-day-old embryos were grown in Eagle minimal essential medium with 2 to 5% fetal bovine serum at 38 C in an incubator with 5% CO2 in air.

Serum neutralization. Serum neutralization was done by plaque reduction against cell-free HVT in chicken embryo cell cultures (10). Chicken serum with unknown titer of neutralizing antibodies was diluted 1:4 in phosphate-buffered saline inactivated for 30 min at 56 C, mixed in equal parts of HVT diluted in SPGA solution (2), to contain about 200 plaque-forming units/0.1 ml, and incubated for 1 h at room temperature. The mixture (0.2 ml) was inoc-
ulated on a semiconfluent monolayer and covered after a 60-min adsorption at 38 C with a 2% carboxymethyl-cellulose overlay. The cultures were washed and stained with 0.1% crystal violet after a 7-day incubation at 38 C in a CO₂ incubator. The number of plaques was determined microscopically, and the serum neutralization effect expressed as X-fold plaque reduction was compared to the control culture.

**Agar-gel precipitation** (4, 12). A microtest of the two-dimensional agar-gel precipitation adopted from the method described by Crowle (5) was used. The agar-gel had the following composition: 1% Noble agar (Difco), 8% NaCl, 1% NaNO₃ in 0.01 M phosphate buffer at pH 7.2. The preparations were stained with azokarmin (1.5 g of azokarmin per liter of acetate buffer, pH 3.7).

**Antigens for gel precipitation.** Avirulent MDHV adapted to chicken embryo kidney cell cultures (MDHV-TC) was used at passage 32 or higher. Infected cells were disrupted by ultrasonic treatment (Sonifier B-12, Branson Sonic Power Co.). Optimal yield of antigen was obtained by sonication (6 × 30 s) with intervals of 1 min under cooling in ice water. The antigen was clarified by centrifugation for 30 min at 12,000 × g and 4 C.

**FF antigen.** FF antigen of MDHV was obtained from soft, vascularized feather follicles of infected chickens cut with scissors, suspended in phosphate-buffered saline, and homogenized with a high-speed blender (Polytron). The suspension was then sonicated for 2 × 30 min under ice cooling and clarified by centrifugation (30 min, 12,000 × g at 4 C).

**RESULTS**

(i) **Relationship between MDHV antigens of tissue culture and FF origin and HVT in gel precipitation.** For this reaction, a potent precipitating serum of chicken vaccinated with HVT on the first day after hatching and challenged with MDHV 2 weeks later was used. The precipitation pattern obtained with immune serum against antigen from MDHV-infected feather follicles, embryonic chicken kidney tissue culture infected with high-passage MDHV-TC, on chicken embryo cell cultures infected with HVT is shown in Fig. 1.

The main precipitation band A obtained against HVT and FF antigen was absent from the MDHV-TC antigen. On the other hand, the strong precipitation band B obtained against MDHV-TC was only weakly present in FF or in HVT antigen. The degree of cross-reactivity between the two main and additional minor antigenic components is shown in Table 1. No reaction to uninfected tissue culture or FF extracts from uninfected chickens was recorded.

Chickens infected experimentally with MDHV reacted independently against one or the other of the two main antigens of FF or MDHV-TC origin.

(ii) **Distribution of FF antigen in MDHV-infected chickens.** In 110 chickens infected with virulent MDHV, the presence of FF antigen was searched for in feather tracts of nine different areas of the skin: back, separately on left and right side of neck, wings, external crural tract, and tail. It was found that the antigen was symmetrically distributed over both sides of the body. Strongest reactions were observed in the large, growing, well-vascularized feather follicles of wings and tail.

The results agree with the assumption that the FF localization of viral antigen is the result of a systemic dissemination of MDHV through the blood vessels.

(iii) **Appearance and persistence of FF antigen.** To study the appearance and persistence of FF antigen and the development of circulating antibody (see below) in MDHV-infected chickens, the following experiments was conducted.

**TABLE 1. Antigenic components of virulent (FF antigen), of tissue culture-adapted avirulent (MDHV-TC) Marek’s disease virus, and of herpesvirus of turkey (HVT) in gel precipitation**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF antigen</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MDHV-TC</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HVT</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Against hyperimmunized chicken serum (see Fig. 1). Symbols: (-) Not detected; (±) (+) weak; (+++) strong precipitation line.
Twenty-eight chickens were immunized immediately after hatching with HVT by i.a. inoculation. Twenty-eight chickens served as unvaccinated controls. At the age of 2 weeks, half of each group were infected abdominally with virulent MDHV. Starting at week 2, two growing feather follicles of each wing were taken at weekly intervals for antigen detection by gel precipitation, and in 2-week intervals serum samples were collected for serological tests. All chickens dying in the course of the experiment were autopsied and examined histologically. Surviving chickens were killed after 13 weeks and examined as above. In addition, direct kidney cultures were done for virus isolation. The following results were obtained.

Unvaccinated chickens. In the 14 unvaccinated chickens infected with MDHV at the age of 3 weeks, FF antigen first became detectable 2 weeks later in 13 of 14 birds. In the nine birds remaining healthy the antigen gradually disappeared. Only two of these nine chickens reacted positively at the age of 13 weeks (Fig. 2). In five chickens that developed the typical disease, however, FF antigen remained present up to the time of death (Fig. 3).

Similar observations were made in a larger group of 186 unvaccinated chickens infected with virulent MDHV in eight experiments. The chickens were killed at various intervals after infection (Fig. 4). Again a significantly different development was found between chickens remaining healthy (clinically and histopathologically) and those that succumbed to MD. Twenty-nine percent of all chickens that died or were killed showed clinical and histopathological signs of MD. Initially 1 to 5 weeks after infection, 8 of 11 diseased and 15 of 17 healthy chickens showed the presence of FF antigen. In the chickens dying from MD between 6 to 15 weeks after infection, more than 80% had FF antigen up to the time of death. In contrast, among the samples of clinically healthy chickens the percentage with FF antigen declined from 89% 1 to 5 weeks after infection to 25% at 16 weeks or later. This decline is highly significant, even if it was taken into account that the sick chickens have been gradually eliminated from the group ($\chi^2, P < 0.01$).

Chickens vaccinated with HVT. None of the 15 chickens immunized with HVT without challenge developed FF antigen. In all of 13 birds from which direct kidney cultures were done at 14 weeks, a persistent infection with HVT was detected. In the 15 vaccinated chickens challenged with virulent MDHV, FF antigen was present in one bird at 2 weeks, in four birds at 3 weeks, and in one again at 5 weeks after infection. No antigen was detectable thereafter, and the chickens remained healthy (Fig. 5).

Control chickens. None of the 15 unvaccinated and unchallenged control birds showed the presence of FF antigen.

(iv) Development of precipitating and neu-
Neutralizing antibody. In the group of vaccinated and unvaccinated chickens mentioned above (Results [iii]), the development of neutralizing antibodies (against HVT) and of precipitating antibodies against MDHV-TC and FF antigens was studied (Table 2 and 3).

Neutralizing antibodies. In uninfected, unvaccinated control chickens a plaque reduction up to 50% was observed with a serum dilution of 1:4. Plaque reduction up to this level was considered nonspecific (Fig. 6).

In chickens vaccinated with HVT with or without subsequent challenge, significant levels of neutralizing antibody developed (Fig. 5 and 6). Antibodies were already detectable 3 weeks after vaccination and gradually increased up to the end of the experiment at 14 weeks of age. One week after challenge with virulent MDHV, the titers in the challenged birds were lower than in the vaccinated controls.

In unvaccinated chickens infected with virulent MDHV, differences in the development of neutralizing antibodies were seen which became significant from week 10 of life until the time of death. Chickens succumbing to MD developed much lower antibody titers than the penmates surviving up to the end of the experiment (Fig. 2 and 3).

In the larger group of unvaccinated chickens mentioned above infected i.a. with MDHV, it was shown that those chickens with persistent MDHV infection or those affected by clinical disease had significantly lower levels of antibodies at the time of death or killing. The results are summarized in Table 3 and 4. Thirteen of fifteen (86%) chickens with no evidence of persistent infection had neutralizing antibodies as compared to only 14 (32%) of those 42 with persistent infections. Similarly only 3 of 16 (18%) chickens coming down with MD had neutralizing antibodies as compared to 25 of 43 (58%) of those without gross evidence of the disease. Both differences are significant (Table 3). Nevertheless, persistent infection was not always associated with clinical disease during the observation period of 14 to 17 weeks.

Precipitating antibodies against MDHV-TC or FF antigens. The results on precipitating antibodies against MDHV-TC or FF antigen are shown in Tables 2 and 3. Again no reactions were found in unvaccinated, uninfected controls.

Vaccination with HVT and/or challenge with virulent MDHV led to the formation of precipi-
TABLE 2. Precipitating antibodies against FF and MDHV-TC antigens

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>10</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0/15a</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/15</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>0/14</td>
<td>0/10</td>
<td>0/10</td>
<td>2/10</td>
<td>5/10</td>
<td>7/10</td>
<td>7/10</td>
<td>10/14</td>
</tr>
<tr>
<td>HVTa</td>
<td>0/14</td>
<td>0/10</td>
<td>2/10</td>
<td>1/10</td>
<td>5/10</td>
<td>7/10</td>
<td>5/10</td>
<td>6/14</td>
</tr>
<tr>
<td>Infected</td>
<td>0/9</td>
<td>0/9</td>
<td>8/9</td>
<td>7/9d</td>
<td>8/8</td>
<td>9/9</td>
<td>7/7e</td>
<td>7/7</td>
</tr>
<tr>
<td>with virulent MDHVc</td>
<td>0/9</td>
<td>3/9</td>
<td>3/9</td>
<td>5/9</td>
<td>5/9</td>
<td>7/9</td>
<td>5/7</td>
<td>7/7</td>
</tr>
<tr>
<td>Surviving</td>
<td>0/5</td>
<td>1/5</td>
<td>3/5</td>
<td>0/4d</td>
<td>4/5</td>
<td>2/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinically</td>
<td>0/5</td>
<td>1/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/4</td>
<td>0/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>affected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>0/15</td>
<td>0/15</td>
<td>12/15</td>
<td>8/15d</td>
<td>11/15</td>
<td>12/15</td>
<td>12/15</td>
<td>13/15</td>
</tr>
<tr>
<td>challenged</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with MDHVc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

a Numerator, number of chickens reacting positively; denominator, number of chickens examined. Six control and four vaccinated chickens were held in uninterrupted isolation during the experiment. The other birds were taken out of the isolators for a few minutes for blood sampling at 1- to 2-week intervals.

b Vaccinated immediately after hatching with HVT.

c Infected with virulent MDHV at the age of 2 weeks.

d The lower percentage of serologically positive birds in many groups at week 7 is unexplained.

e Two chickens were lost by causes other than MD.

TABLE 3. Formation of humoral antibodies in chickens with or without persistent MDHV infection

<table>
<thead>
<tr>
<th>Humoral antibodies</th>
<th>(1) Chickens with persistent MDHV infectionb</th>
<th>(2) Chickens without persistent MDHV infectionb</th>
<th>Comparison of significance (1) vs (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>antibodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF antigen (A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>37</td>
<td>15</td>
<td>No significant difference</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>1</td>
<td>(χ², P &gt; 0.25)</td>
</tr>
<tr>
<td>MDHV-TC antigen (B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>17</td>
<td>13</td>
<td>Difference is highly significant</td>
</tr>
<tr>
<td>Negative</td>
<td>28</td>
<td>3</td>
<td>(χ², P &lt; 0.01)</td>
</tr>
<tr>
<td>Neutralizing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>antibody</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positivev</td>
<td>14</td>
<td>13</td>
<td>Difference is highly significant</td>
</tr>
<tr>
<td>Negative</td>
<td>28</td>
<td>2</td>
<td>(χ², P &lt; 0.002)</td>
</tr>
</tbody>
</table>

a Unvaccinated chickens infected intra-abdominally with virulent MDHV at 1 to 3 weeks of age.

b Persistent infection with FF antigen detectable at time of death or killing and/or MDHV present in direct kidney culture at the end of the experiment at the age of 3 to 4 months.

c Plaque reduction at serum dilution (1:4) greater than 50%.

tating antibodies against both FF (component A) and MDHV-TC (component B) antigens in a large proportion of chickens, with the exception of one group: chickens clinically affected with MD developed antibodies against the FF antigen, but developed little, if any, antibody against the MDHV-TC antigen.

A similar relationship was found in the larger collection of unvaccinated birds from several different experiments after i.a. infection with virulent MDHV. Among 66 unvaccinated chickens, 86% developed antibody against FF antigen, but only 49% developed antibody against MDHV-TC antigen. Precipi-
tating antibodies against FF antigen (A) were found with equal frequency in chickens with persistent MDHV infection (FF antigen or kidney culture positive at time of killing) and in those in which virus or viral antigen became undetectable. Against MDHV-TC antigen (B), however, the percentage of chickens with antibodies was significantly lower in those with persistent infection of the feather follicles than in those without persistent infection (Table 3).

In chickens with gross evidence of MD, only 2 of 18 were forming precipitating antibodies against MDHV-TC, in contrast to many as 30 of those 47 without the disease. This difference is highly significant. The formation of precipitating antibodies against FF antigen was also reduced, but much less so.

![Image](http://iai.asm.org/)

**FIG. 6.** Formation of neutralizing antibodies in HVT-vaccinated chickens without challenge. Lack of antibody formation in unvaccinated chickens without challenge.

<table>
<thead>
<tr>
<th>Humoral antibodies</th>
<th>(1) Chickens affected by Marek’s disease</th>
<th>(2) Chickens with no gross evidence of Marek’s disease</th>
<th>Comparison of significance (1) vs (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF antigen (A)</td>
<td>Positive</td>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>MDHV-TC antigen</td>
<td>Positive</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Neutralizing antibody</td>
<td>Positive</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>13</td>
<td>18</td>
</tr>
</tbody>
</table>

*a* Unvaccinated chicken infected intra-abdominally with virulent MDHV at 1 to 3 weeks of age.

*b* Clinical signs of Marek’s disease were confirmed by autopsy and histopathology. Experiment was terminated at the age of 3 to 4 months.

*c* Plaque reduction at serum dilution (1:4) greater than 50%.

**DISCUSSION**

Eighty to one hundred percent of the chickens infected i.a. with virulent MDHV at 0 to 2 weeks of age showed as early as 2 weeks later a generalized infection of the feather follicles, as detected in our studies by gel precipitation as described by Haider et al. (7). The distribution of infected feather follicles more or less symmetrically over the whole skin suggests an early spread of the virus by the blood circulation.

The further development of FF infection appeared to be correlated with the fate of individual birds. Chickens succumbing to the disease showed in about 80% a persistent infection of the feather follicles up to the time of killing or death. An association between persistent viremia and MD lesions was also found by Witter et al. (24) in a long-term study of a naturally infected chicken flock. In infected chickens remaining clinically healthy in our study, however, the FF antigen, which was initially present in the same proportion as in those which eventually died, slowly disappeared. This disappearance is real and is not only due to the fact that MD-sick chickens were gradually eliminated from the group.

The early generalization of MDHV may be responsible for inflammatory neural lesions without clinical signs, which we found in about 85% of the infected chickens between 1 to 5 weeks after infection. In about 30%, however, these lesions gradually disappeared up to and after week 16 after infection. (Steck, unpublished data). This may be the same manifesta-
tion of MDHV infection as the temporary paralysis observed by Kenzy et al. (9). The suppression of the generalized infection in many birds may reflect the age-related resistance observed by others (19, 22).

Infection with MD leads to the formation of antibodies against several different viral antigens. We followed the development of precipitating antibodies against FF antigen (antigenic component A), MDHV-TC antigen (antigenic component B), and neutralizing antibodies against HVT. It appears important to differentiate between antibodies against various antigens, since the antibody response against those three antigens is not going in parallel and only the last two mentioned appeared to be correlated with resistance against disease or recovery.

Most, i.e., 86% of the birds infected with virulent MDHV or HVT, reacted with the formation of precipitating antibody against the main antigenic component A or FF antigen, irrespective of whether the virus infection persisted. In chickens with clinical MD the antibody response was significantly lower than in healthy chickens. Since, however, as many as two-thirds of the sick chickens had antibodies against the FF antigen up to the time of death, this antibody does not seem to be effective in resistance against disease, in accordance with the results obtained by Sharma and Stone (18).

Neutralizing antibodies, measured against HVT, and precipitating antibodies against the antigenic component B of MDHV-TC were less readily detectable in all birds than the antibody against FF antigen mentioned above. However, they were more frequently seen in birds which remained clinically healthy or where the infection did not persist. Sharma and Stone (18) and Calnek (1) suggested that the formation of virus-neutralizing antibodies may be involved in the genetic resistance of chickens against MD. The situation is, however, not quite the same in respect to persistent infection with MDHV or to clinical manifestation of infection.

Most birds with no evidence of persistent MDHV infection showed neutralizing and precipitating (MDHV-TC) antibodies; only a few were without. However, about a third of those with persistent infection had also neutralizing or precipitating (MDHV-TC) antibodies. Since the experiments were terminated at the age of 14 to 17 weeks, it is not possible to know whether these birds would have overcome the generalized infection at a later stage.

In the birds with clinical and pathological evidence of MD the formation of antibodies was reduced. This has also been found to be true against antigens unrelated to MDHV (11, 16). In particular, only a small fraction of these birds had precipitating antibodies against MDHV-TC (component B) or neutralizing antibodies. In contrast, about two-thirds of the surviving chickens had both antibodies. The difference between healthy chickens and those affected clinically by MD is highly significant. It was, however, not possible to establish a direct causal relationship. The two mentioned antibodies did not go strictly parallel in each bird and are probably directed against two different antigenic components, but they both seem to reflect an immune status of the bird which tends to overcome the persistent generalized infection and possibly also the development of irreversible lesions. It has been shown by others that bursectomy or thymectomy had no effect on the development of MD (13) in contrast to lymphoid leukosis, where removal of the bursa Fabricii prevents the development of the disease (14). We found in unpublished experiments that the combination of surgical removal of the thymus before and of the bursa Fabricii immediately after hatching almost completely suppressed the formation of circulating antibodies and led to a persistent infection in all birds. We found, on the other hand, no evidence that the antibodies studied were directly involved in the pathogenesis of lesions.

The immunization of chickens with live HVT did lead, in agreement with the results obtained by others (6, 17), to a persistent infection with the vaccine virus, but without localization in the feather follicles detectable by gel precipitation. The vaccinated birds developed, as after infection with MDHV, precipitating antibodies against MDHV-TC antigen, FF antigen, and neutralizing antibodies.

In immunized birds the challenge infection with virulent MDHV led to a generalization only in a few birds. The localization in feather follicles, if it did occur, appeared to be only transitory. They were protected against clinical disease.

It has been shown by Kaaden and Dietzschold (8) that MDHV-infected cells acquire virus-specific proteins in their plasma membrane detectable by immunodiffusion and indirectly detectable by virus neutralization. At least one of the most likely virus-induced components of MDHV-infected chicken embryo fibroblasts stimulated the formation of cross-neutralizing antibodies against HVT.

It is conceivable that the neutralizing or precipitating (against MDHV-TC) antibodies in our experiments associated with the regression of lesions or the suppression of a generalized infection of feather follicles are not, or only in part, directly effective by neutralizing free vi-
rus. They may also interact with specific antigens located on the surface of virus-infected cells. In addition, the role of cell-mediated immunity in MD is still not elucidated. Since the elimination of virus-infected cells is probably less efficient than the elimination of free virus, this would explain in part why the correlation between recovery and antibody formation is not very strict.

In summary, chickens were infected with virulent MDHV with or without prior immunization with HVT.

The sequence of infection was followed by studying the humoral immune response and the appearance of precipitating antigen in feather follicles (FF antigen). It was differentiated between precipitating antibodies against MDHV-FF antigen and against tissue culture-adapted MDHV and neutralizing antibodies tested against HVT.

Beginning about 2 weeks after infection with virulent MDHV 80 to 90% of all unvaccinated chickens showed a generalized infection of feather follicles, which regressed again in about one third of all chickens. The prior immunization with HVT suppressed this localization of infection to a large extent.

In most chickens succumbing to MD the infection persisted in the feather follicles up to the time of death, and only a small fraction of these birds had precipitating antibodies against MDHV-TC or neutralizing antibodies.

On the other hand, most of the chickens with no evidence of persistent infection developed precipitating antibodies against MDHV-TC and neutralizing antibodies.

In between was a group of chickens with persistent infection with or without antibodies but with no evidence of MD. Since the experiments were terminated after observation periods of 3 to 4 months, it is unknown whether these birds would eventually have come down with the disease or would have overcome the infection depending on their immune status.

In contrast to precipitating antibodies against MDHV-TC and neutralizing antibodies, which appeared to reflect resistance to infection and disease, the precipitating antibodies against FF antigen were seen in most birds regardless of their fate.

ACKNOWLEDGMENTS

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LITERATURE CITED


