Immunofluorescent Studies of Lymph Nodes and Spleens of Leukotic Cattle for Cells Producing IgM and IgG

Z. Trainin and U. Klopfer
Kimron Veterinary Institute, Post Office Box 12, Bet Dagan, Israel

SUMMARY

Lymph nodes and spleens of 31 leukotic cattle were subjected to immunofluorescent testing to ascertain whether they contained IgM- and IgG-producing cells. The tests indicated that the comparatively marked reduction of IgM in the serum of leukotic cattle was due to a decline in the production of IgM in the spleen and lymph nodes. In the lymph nodes concerned, no IgM-producing cells were found, while 3 to 5 IgG-producing cells per field were present. It appears, therefore, that the damage to the lymph system of leukotic cattle is selective and primarily affects the IgM-producing cells. The findings also indicate that the damage to the IgM-producing cells seems to occur before the morphological changes are visible.

INTRODUCTION

Since bovine leukosis is a lymphoproliferative disease, there are grounds for assuming that it is accompanied by immunological changes. The possibility of immunological changes in the sera of leukotic cattle has already been investigated by Nadim (4) and Mathaeus and Straub (3), who tested such sera by paper and gel electrophoresis. These methods showed no significant changes in the blood proteins of leukotic cattle. By means of immunoelectrophoresis, however, it has been shown that the blood serum of leukotic cattle lacks a specific protein (5), IgM (6).

Examination of the serum of cattle in which the presence of the disease has been proven histologically disclosed that in about 80% IgM was absent or present only in traces, while in about 20% IgM occurred in normal or greater than normal quantities (7, 8). This research led to the conclusion that the absence of IgM in the serum of leukotic cattle is pathognomonic for bovine leukosis (8).

Klopfer and Trainin (2) have described a case of bovine leukosis in which the disappearance of IgM preceded the development of tumors. The process was also found to be reversible; i.e., the IgM may, if only briefly, reappear in the blood in normal quantity (2).

In view of these findings, the question arises of whether the complete or partial disappearance of IgM from the serum of the leukotic bovine is due to the cessation of the production of this immunoglobulin in the lymphatic system or whether the lymphatic system produces IgM normally and its disappearance from the blood circulation is due to some other cause. To settle this question, we examined lymph nodes and spleens of diseased cattle by immunofluorescence to ascertain whether these organs contained IgM-producing cells. A preliminary paper (7) has described a number of cases which indicate a direct connection between the IgM level in the serum and the presence or absence of cells producing this immunoglobulin in the lymphatic system.

A summary of the research performed on this subject follows.

MATERIALS AND METHODS

Animals. Tumorous lymph nodes, lymph nodes of normal appearance, spleens, and sera removed at slaughtering or after death from 31 cattle proved to have spontaneous bovine leukemia on histological examination. The material was prepared while fresh.

Preparation of Tissue Sections. Touch preparations were prepared from cuts freshly made in the organ. The preparations were air dried for a few min, then fixed in acetone for 30 min at room temperature, and immediately frozen at —20° until they were stained.

Preparation of Pure Bovine IgG. Forty ml of diethyaminoethyl Sephadex A-50 suspended in phosphate buffer, pH 7.6, 0.02 M, were added to 10 ml of cattle serum. The material was mixed overnight and then transferred by suction through a glass filter. The process of mixing and separation was repeated. The liquid obtained was tested by immunoelectrophoresis and the Ouchterlony test and found to be pure IgG.

Preparation of Bovine Anti-IgG. Pure cattle IgG was injected into rabbits with complete Freund's adjuvant according to the method described by Fey (1). The rabbit serum anti-bovine-IgG was absorbed by adding bovine IgM (which was not pure and also contained a-macroglobulin) in the ratio of 0.2 ml of IgM to 10 ml of rabbit serum anti-bovine-IgG.

Preparation of Bovine Anti-IgM. This was performed by the method described in another paper (6). The anti-IgG and the anti-IgM sera were tested by the Ouchterlony technique against bovine serum. The anti-IgG and anti-IgM did not show cross-reaction.

Preparation of Bovine Anti-IgM and Anti-IgG Fluorescent Conjugate. Twenty ml of anti-IgM or bovine anti-IgG rabbit serum were precipitated in 50% ammonium sulfate. The precipitate obtained after 18 hr was suspended twice in 40% ammonium sulfate and then dissolved in 10 ml of distilled...
The solution was dialyzed against 0.9% NaCl solution for 72 hr. After dialysis, the protein in the solution was measured by the biuret method. To the solution, fluorescein isothiocyanate (Bioquest, BBL Division, Cockeysville, Md.) was added in the proportion of 1:40 (1 mg of fluorescein isothiocyanate to each 40 mg of serum protein). After this was mixed for 10 min in a magnetic stirrer, about 2 to 4 ml of carbonate buffer, pH 9.7, were added until the pH of the solution reached 9.5. The solution was mixed for 16 hr at 4° and then centrifuged at 12,000 rpm for 30 min. The liquid was separated and purified in a column of G-25 Sephadex pH 7.6, 0.01 M and then concentrated to 5% of its original volume by vacuum dialysis.

Five ml of the concentrate were introduced in a column of diethylaminoethyl Sephadex A-50 suspended in phosphate buffer, pH 7.3, 0.01 M (20 x 0.9 cm) for 16 hr and then rinsed 3 times (H. Fey, personal communication) as follows: 1st rinse with pH 7.3, 0.01 M phosphate buffer + 0.03 M NaCl; 2nd rinse with pH 7.3, 0.01 M phosphate buffer + 0.05 M NaCl; and 3rd rinse with pH 7.3, 0.01 M phosphate buffer + 0.28 M NaCl. After each rinse, the liquid was collected separately. The liquid of the 3rd rinse was concentrated by vacuum dialysis to 5 ml. The conjugated anti-IgM and anti-IgG were tested immuno electrophoretically with bovine serum and were found to be specific. The material was tested on touch preparations of normal spleen. The conjugate was diluted as follows: 1 : 2, 1 : 4, 1 : 8, 1 : 16, 1 : 32, and 1 : 64. One unit was determined to be the last dilution with a detectable fluorescence. Four units were used experimentally.

The anti-IgM conjugate was found suitable for use at 1 : 8 dilution, and the anti-IgG conjugate was found suitable for use undiluted. To the conjugate, Merthiolate in a concentration of 1 : 10,000 was added.

Staining of the Preparations with the Conjugates. The touch preparations were covered with 1 drop of conjugate and kept in a moist chamber at room temperature for 30 min. Then the preparations were rinsed 3 times with phosphate-buffered saline, each time for 10 min. The sections were air dried and covered with a coverglass with glycerine and phosphate-buffered saline (in a ratio of 1 : 10). Examination of the preparations by fluorescent microscope was carried out immediately after staining.

All tests of leukotic bovines by the immunofluorescence test were run together with lymph nodes and spleens from normal cattle. Determination of the specific fluorescence and its degree was performed by the blind method; i.e., the person performing the test did not know what the origin of the material was and whether it had been taken from a diseased or healthy animal.

RESULTS

The results of the direct immunofluorescent test with anti-IgM and anti-IgG conjugate from spleens, tumorous lymph nodes, and "normal" lymph nodes of 31 leukotic bovines are shown in Tables 1 and 2.

Table 1 contains data on 25 cattle in the blood of which IgM was not found or found only in traces. No cells containing IgM were found in the tumorous lymph nodes of any of these bovines, or in the spleen and the normal-appearing lymph nodes of 25 leukotic cattle with no traces of IgM in their sera.

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Table 2 presents data on 6 leukotic cattle in the blood of which IgM was found in the tumorous lymph nodes of any of these bovines, or in the spleen and the normal-appearing lymph nodes, although traces of IgM could be seen. In all those organs it was, in general, possible to find more than 3 cells per field containing IgG. Only part of the tumorous lymph nodes showed a reduction in the number of cells containing IgG.

Table 2 presents data on 6 leukotic cattle in the blood of which IgM was found. In the tumorous lymph nodes, no IgM-producing cells were visible, but they existed in considerable quantity in the spleen and the normal lymph nodes. In these bovines, cells containing IgG were found in all the organs concerned. Lymph nodes and spleens from normal cattle showed moderate to very strong fluorescent cells for IgG and IgM.

DISCUSSION

In the immunofluorescent tests performed on tumorous lymph nodes of leukotic cattle, whether their blood lacked IgM or contained it in normal quantities, IgM-producing cells were absent. Moreover, even in the normal lymph nodes and spleens of cattle in which the blood lacked IgM, virtually no IgM-producing cells were found. These findings clearly indicate that the lack of this immunoglobulin in the blood of leukotic
cattle is due to a considerable reduction of IgM production in the spleen and the lymph nodes.

On the other hand, the number of IgG-producing cells in the spleens and normal lymph nodes was high. Only in the tumorous lymph nodes was there some reduction but, even there, activity could still be established in a considerable percentage of the cases, which explains the normal amounts of IgG in leukotic cattle. It follows, therefore, that the damage to the lymphatic system of leukotic cattle is selective and affects the IgM-producing cells.

The fact that, on the one hand, even the normal lymph nodes lack IgM-producing cells and, on the other hand, the IgM cannot be found in the blood of leukotic cattle even before the tumors are formed (2) gives grounds for the assumption that the damage to the IgM-producing cells occurs before the visible morphological changes appear. It can be assumed that the disappearance of the IgM is not necessarily the result of the morphological changes in the lymph nodes and spleen. However, the possibility that the pathogenic agent directly and specifically attacks the IgM-producing system exists.

Study of the connection between the disappearance of the IgM and the pathogenesis of bovine leukemia could contribute to the understanding of the leukomogenous process in this disease.

REFERENCES

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