Relationship between formaldehyde-related antibodies and cross-reacting anti-N-like antibodies in patients undergoing chronic haemodialysis

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SUMMARY An attempt was made to determine the sequence of events leading to the production of two distinct antibodies in patients with chronic renal failure who regularly undergo haemodialysis with formaldehyde-resterilisable dialysis units by Multipoint (UK) or Cordis (USA). A distinct pattern emerged—namely, the production of anti-formaldehyde red cells started about six months after the beginning of haemodialysis treatment. Only when the titre of these antibodies reached 64 or 128 another, apparently cross-reacting, antibody appeared which reacted like an anti-N antibody. A strong direct antiglobulin reaction was found to be positive for formalin-treated red cells after five minutes' contact with the specific antibody, indicating a high affinity of the antibody to the formalin-altered red cell.

Haemodialysis as a means of saving the lives of patients suffering from chronic renal failure is an every day event. The problems arising in some cases were not, however, realised until the first publication by Howell and Perkins in 1972,¹ which has since been followed by several others.²⁻⁸ The problems at first seemed to be merely serological, causing some inconvenience to the blood bank laboratory trying to find units of blood compatible with the patient's unexpectedly produced antibodies. Since then the scope of the problem has widened and become an immunological one.

Howell and Perkins¹ first described the formation of what they termed 'anti-N-like antibodies' in the sera of patients undergoing chronic haemodialysis. Their paper speculated only on the possible reason for the production of antibodies, suggesting possible mechanical damage to the red cells while in touch with the dialysis membrane. In 1977 Gorst *et al.*⁹ described what they termed 'formaldehyde-induced anti-N'. They first named the formaldehyde, which was applied at a concentration of 6% to resterilise the haemodialysis membrane, as a possible cause of the alteration of the human red cells during their contact with the formaldehyde-sterilised membrane—an alteration that made those cells immunogenic to the patient in what seemed to be an autoimmune response. The concept of autoimmunity was based

on the fact that some of the patients who had developed anti-N-like antibodies were homozygous for N, while some of the rest were heterozygous—that is, MN. The only disadvantage of their experimental procedure to imitate what may, in fact, happen to the patient was the long contact between the red cells and formaldehyde that it entailed.

Sandler *et al.*^{10,11} distinguished two antibodies formed by patients undergoing chronic haemodialysis. These were produced only by patients being treated with formaldehyde resterilisable dialysis membranes and not by those treated by other dialysis procedures, and production was not related to chronic renal failure in itself.

The aim of our investigation was to elucidate, firstly, the sequence of events in the production of these antibodies, and, secondly, whether the production of one antibody is dependent on the other.

Patients and methods

The sera to be tested were collected over a period of five years from 25 patients suffering from chronic renal failure regularly undergoing haemodialysis three times a week. All patients in this group were treated with resterilisable dialysis membranes by Multipoint (UK) or Cordis (USA). The sera were collected and frozen at -40°C . Red cells used for the various tests were (1) human O MM cells; (2) Selectogen (Ortho Diagnostics, New Jersey); and

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(3) sheep red cells. Formaldehyde (Sigma) was diluted with saline to a concentration of 1%.

Human OMM red cells, as determined by commercial antibodies anti-M and anti-N (Ortho, New Jersey) were washed three times, packed to a haematocrit of 50, and an equal volume of a 1% formaldehyde solution was added. The mixture was incubated for 30 minutes at 37°C with continuous agitation. After that the red cells were washed three times, or until supernatant was no longer haemolytic, and resuspended into a 5% suspension.

The sera were tested for the presence of anti OMF (anti-F) as well as for anti-N-like antibodies by incubating equal volumes—that is, two drops of serum with two drops of a 5% suspension of red cells—for 30 minutes at room temperature and the reaction was determined after 30 seconds' spin at 3400 rpm in a Clay Adams serofuge. The strength of the reaction varied from 1+ to 4+. Absorptions were performed at room temperature since this was optimal for both antibodies detected.

Tests applying sheep red cells were performed only after the patient's serum was absorbed with normal sheep red cells. Treatment of sheep red cells with formaldehyde was the same as the treatment of human red cells.

Results

The sera of 25 patients undergoing chronic haemodialysis (two to three times a week) were tested for the presence of anti-F as well as for the presence of anti-N-like antibodies. All sera that reacted with OMF cells were also titrated with the same cells. To determine the sequence of the production of the two antibodies all sera were tested, beginning with samples collected in 1975 up to the present. Eight typical samples are shown in the Table. As can be seen, no anti-N-like antibodies were detected so long as there was either no anti-F activity or until the titre of anti-F was above 64 or 128.

The relationship or interdependence of the antibodies was determined by absorption experiments.

Difference in anti-F titre and the relation of anti-N-like antibodies to the titre of anti-F

Case No.	No. of years of dialysis	Anti-formaldehyde titre	Presence of anti-N-like antibodies
1	4	8	—
2	4	16	—
3	4	64	—
4	5	8	—
5	7	32	±
6	7	256	3+
7	7	2048	4+
8	12	4096	4+

Removal of anti-N-like antibodies by absorption with ONN cells did not affect the activity of anti-F antibodies. A further test was performed by applying sheep red cells treated with a 1% formaldehyde solution. The sera to be tested were first absorbed with normal sheep red cells, then a second absorption was carried out with F sheep red cells. This absorption of the sera with formalin-treated sheep red cells reduced the activity present in the patient's serum against OMF cells. Further experiments showed that if antibody titre against OMF cells was reduced by repeated absorptions with either human or sheep red cells treated with formaldehyde to a titre below 64 the anti-N activity completely disappeared.

Discussion

These findings confirm the association of anti-N-like antibodies with chronic haemodialysis in patients treated with formaldehyde-resterilisable dialysis units. They also indicate that the relation between two antibodies produced by some of the patients undergoing chronic haemodialysis is time-dependent, in the sense that anti-F production began only after a period of six months to one year of haemodialysis treatment. Only when the patient remained on the same procedure of treatment (that is, resterilisable dialysis units) did the production of anti-F antibodies continue, both in terms of strength of reaction (reaching a maximum of 4+ as opposed to 1+ when anti-F antibodies first appeared in the serum) and of continuous rise in titre of antibodies. Anti-N-like antibodies could first be detected when the titre of anti-F antibodies increased above 64 or 128. This sequence can be explained by our previous work^{10 11} describing two events related to the presence of traces of formaldehyde in the dialysis units that affects both the antigenicity of the red cells and the antibody response to various antigens.¹²

We assume that production of antibody begins only after the prolonged stimulation of the immune system by the F-altered red cells, or possibly it may start long before it can be detected. It is clearly a true antibody because of its specific reaction with monospecific anti-IgG serum.¹⁰ The fact that the antibodies reacting with the formalin-treated red cells are IgG antibodies and yet the reaction takes place at room temperature, and the fact that the reaction occurs within five minutes or less probably indicate that the formaldehyde neutralises the negative charge of the red cells. This could explain why the IgG antibodies are able to cause direct agglutination without the aid of antiglobulin serum. Anti-N-like antibodies, if they are antibodies, seem to be a late appendix to the anti-F antibodies, which may not have any effect on the patient.

The most probable explanation for the production of two distinct antibodies resulting from the use of formaldehyde resterilisable dialysis membranes is that the immune response of the patient is triggered by a formaldehyde alteration of a protein which is familiar yet not identical with the glycoprotein that defines the M and N antigens. As a result, antibodies are first formed against this new immunogen (formalin-altered protein). On further contact with that protein the titre of the antibodies rises yet the specificity of the antibody is lowered and it starts cross-reacting with the native N antigen to form the second antibody, the anti-N-like antibody.

There has as yet been no report of an autoimmune haemolytic anaemia related to autoanti-N-like antibodies—which is what these antibodies in most cases are, since anti-N-like antibodies were found in homozygous NN patients, as well as heterozygous MN.⁹ Further investigations will have to be performed in animals to establish the relationship between the production of anti-F and the apparent 'autoantibody', so far termed anti-N-like antibody.

The fact that formalin-treated sheep red cells absorb an anti-F activity present in the serum indicates that the anti-F activity is not species-specific, so that the change caused by formaldehyde to a protein constituent of the red cell membrane must be specific for the red cell yet not a human specific antigen.

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