Immunofluorescence of HL-A Antigens in Kidney Tissue

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ABSTRACT

HL-A antigens can be demonstrated on kidney cells by means of the immune adherence technique.

In this study we have found that HL-A antigens can also be demonstrated in kidney tissue by means of the immunofluorescence technique. An advantage of this method is the possibility of determining the localisation of HL-A antigens. In kidney sections we found the following patterns:
1. Deposits along the endothelium of the glomerular basal membranes
2. Deposits along the endothelium of the arteries
3. Deposits in the interstitium
4. Deposits along the tubule cells.

The first two types of deposits were always found, the third less frequently, whereas the fourth was relatively rare.

The localisation of HL-A antigens may explain why rejection starts at the endothelium of the glomerular basal membranes and the endothelium of the arteries.

The indirect immunofluorescence technique can only be applied with confidence to kidney sections without immunoglobulin deposits. The direct method offers the possibility of detecting HL-A antigens when immunoglobulin deposits are present.

HL-A typing of kidney tissue by the immunofluorescence method and HL-A typing of lymphocytes by the lymphocytotoxicity test gave identical results in 99% of the cases.

INTRODUCTION

HL-A antigens have been demonstrated on kidney cells by means of the micro immune adherence assay (de Planque et al, 1969; Pierce & Hume, 1971; Sybesma et al, 1973), the fluorochromasia cytotoxicity technique
(Perkins et al, 1970) and by means of absorption studies (Verrier Jones & Moore, 1971). HL-A typing of kidney cells and lymphocytes from the same donor does not always yield identical results.

We studied the presence of HL-A antigens on kidney sections by means of the immunofluorescence technique while the lymphocytes from the same individuals were typed by means of the cytotoxicity technique. We undertook this study for two reasons: to compare the HL-A typing results of kidney tissue and lymphocytes and to study the localisation of HL-A antigens in kidney tissue.

MATERIALS AND METHODS

Fresh kidney tissue was obtained by needle biopsy or at nephrectomy. A portion was used for this study while the remaining tissue was processed for routine histological examination.

Lymphocytes were typed by means of the microcytotoxicity test as described by Kissmeyer Nielsen and Kjerbye (1967).

The presence of HL-A antigens on kidney sections was studied by means of the indirect immunofluorescence technique (Coons, 1954 and 1956), using human anti-HL-A sera, rabbit anti-human sera (anti-IgG, anti-IgM and anti-IgA) and fluorescein conjugated horse anti-rabbit serum.

The anti-HL-A sera used in this study have been described elsewhere (Sybesma et al, 1973). The specificity of the immunofluorescence was studied by using AB serum as a negative control and a poly-specific anti-HL-A serum as a positive control. The AB-serum has been screened for the absence of cytotoxic antibodies against lymphocytes and for the absence of antibodies against kidney cells by means of the micro immune adherence assay as previously described (Sybesma et al, 1973).

Other controls consisted of direct application of the rabbit anti-human sera on the kidney sections, followed by the fluorescein conjugated antibody and of direct application of the fluorescein conjugated antibody only.

Only kidney sections on which these control tests were negative were used for the demonstration of HL-A antigens in kidney tissue.

RESULTS

Using the immunofluorescence technique for the demonstration of HL-A antigens in kidney sections the following patterns were found:

1. Deposits along the endothelium of the glomerular basal membranes (GBM).
2. Deposits along the endothelium of the arteries.
3. Deposits in the interstitium.
4. Deposits along the tubule cells.
The first two patterns were found in all kidneys. They are shown on Figures 1 and 2. Deposits in the interstitium were found less frequently and showed only weak fluorescence. Localisation on the tubule cells was found to be relatively rare.

In 34 out of 35 cases an HL-A antigen that had been found on the individual’s lymphocytes could also be demonstrated in the kidney. In all 44 cases where the reaction of a given anti-HL-A serum with the lymphocytes was negative, the immunofluorescence test on kidney tissue also yielded a negative result.

Figure 1. Depositions of HL-A antibodies along the endothelium of the GBM

Figure 2. Depositions of HL-A antibodies along the endothelium of the arteries
DISCUSSION

The distribution of HL-A antigens in the kidneys studied is mainly on the endothelium of the GBM and the arteries. The deposits along the endothelium were of a patchy and interrupted linear character. In the interstitium and on the tubule cells deposits were found less frequently, while the exact localisation was hard to determine.

During rejection of kidney transplants deposits of immunoglobulin and complement have been found along the endothelium of the GBM and the arteries (Andres et al., 1970; MacKenzie & Whittingham, 1968). Such deposits have also been found in transplanted kidneys of patients without clinical rejection (Go, 1972). However, in patients with rejection they are found more frequently (MacKenzie & Whittingham, 1968).

Thus it appears that IgG and complement are deposited at exactly the same place as the greatest density of HL-A antigens. This suggests that in transplanted kidneys humoral antibodies interact with the HL-A antigens of the donor kidney and thus may play an important role in rejection. Porter (1967) suggests that interaction of humoral antibody with an antigen in vessels leads to complement fixation, clumping of platelets and formation of a thrombus with deposition of fibrin. This eventually would cause occlusion of vessels with impairment of kidney function due to ischaemia.

The advantage of the indirect immunofluorescence technique over other methods for demonstrating HL-A antigens on kidney cells, is the possibility it provides for study of the distribution of HL-A antigens in kidney tissue. However, blocking procedures are not possible with this method and furthermore it cannot be applied on kidney tissue with pre-existent depositions of immunoglobulins.

The direct immunofluorescence technique lacks both these disadvantages and therefore seems to be the method of choice. A practical drawback is the necessity of labelling a large number of anti-HL-A sera with fluorescein. Our preliminary results with this method confirm the results obtained with the indirect technique.

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OPEN DISCUSSION

F P BRUNNER (Basel): I am not quite clear: were these sections taken
from donor kidneys before transplantation or were they all from routine
kidney biopsies?

SYBESMA: Both. We have used donor kidneys that have not been trans-
planted since as I have already said you can only use kidneys that have no
immunoglobulins in them for this procedure. The controls are very impor-
tant, and you use this technique only when the difference is easily visible.
It cannot be used for patients that have already been transplanted.

van BREDAB-VRIESMAN (Leiden, Holland): I believe that your Group showed
some years ago that the antigens are on the renal tubular cells. Now you
are getting different results using a different technique. Do you have any
explanation for this? Secondly, it is well known that in vivo the antigens are
not only distributed on the endothelium of the glomerular arteries, but also
to a great extent in the tubular capillaries. Where are the antigens on the
tubular capillaries?

SYBESMA: Answering your last question first I would state that the antigens
are present on all arteries. They are generally seen better on small rather
than large arteries. Regarding your first question, I had heard that your
Group was showing the HL-A antigens on the tubular cells. I cannot say that
they are not present on tubular cells, but they are more difficult to see. If
you compare tubular cells tested with rabbit serum, using anti HL-A sera
and AB serum, which has been screened for the absence of cytotoxic
antibodies and kidney specific antibodies, you will then be able to tell the difference, although you will not be able to see a specific deposit.

J H GO (Nymegen, Holland): Dr Sybesma I should like to congratulate you on your paper. I do not think that the slide you showed of the immunofluorescence was quite clear since there was also mesangial fluorescence. I should also like to know whether you consider the fluorescence along the basement membrane to be of granular or of the short linear type – that you previously described in 1970?

SYBESMA: I think that your question is best answered by my showing you some more slides. On the first slide I am sure you can see the thickening with a granular pattern: there is no doubt about this. The second slide shows thickening with a linear deposition, and the third shows a possible questionable break in the deposition along the endothelium: in this instance the deposition is neither granular nor linear.