IMMUNOGENETICS AND IMMUNOPATHOLOGY OF HUMAN MEMBRANOUS GLOMERULONEPHRITIS

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Summary

In most of our membranous glomerulonephritis (MGN) patients, we have studied the HLA-A, B, DR phenotype, the clearance of anti-D rhesus coated ^{51}Cr-labelled autologous erythrocytes, and T-lymphocyte subpopulations with monoclonal antibodies (OKT3, T4, T8). The frequency of B8 antigen in 28 patients with MGN (five cases associated with lupus excluded) is 57.14 per cent versus 14.42 per cent in controls (Pc=0.0002). In 26 patients the frequency of DR3 antigen is 65.38 per cent versus 20.27 per cent in controls (Pc=0.00008). The half-life of sensitised erythrocytes is respectively 35.36 ± 8.50 minutes in nine controls and 67.05 ± 69.64min in 18 patients with MGN. The half-life is significantly prolonged in one-third of the patients at time of exacerbation. The peripheral blood T-lymphocyte subsets study showed a significant decrease of OKT3 and OKT4 positive cell subsets. T4/T8 ratio is high during exacerbation of disease and diminishes in remission. Our data are consistent with a latent subtle genetic immunodeficiency, only expressed during acute phases of the disease.

Introduction

Membranous glomerulonephritis (MGN) is thought to be HLA linked, involving B8 or/and DR3 antigens [1]. Recent work [2] suggests that B8 DR3 positive subjects have an abnormal immune response involving Fc receptor dysfunction on macrophages and on T (Fcγ) cells.

In this work we studied the HLA typing and T-cell subsets in our patients with membranous glomerulonephritis.

Material and methods

The patients

Between January 1976 and December 1981 40 patients have been diagnosed on renal biopsy as MGN. Five patients had systemic lupus, and the remaining 35 were considered as primary MGN.

HLA typing

HLA-A, B, DR antigens were determined by classical techniques using France-Transplant antisera. Twenty-eight Caucasians with primary MGN were typed for HLA-A, B and 26 for HLA-DR.

Clearance of autologous labelled and sensitised erythrocytes

The technique is adapted from Frank et al [3]. The rhesus positive red blood cells are 51-chromium labelled, sensitised with pure anti-D rhesus human IgG, and then reinjected. Half-life is calculated from samples obtained 10, 20, 30, 45 and 60 min after reinjection.

We studied nine normal volunteers, two splenectomised patients, one with polycythemia vera and 18 primary MGN.

Peripheral blood T- lymphocyte subsets

The total T-lymphocytes, the helper T-cells and the suppressor T-cells percentage have been studied by OKT3, OKT4 and OKT8 mouse monoclonal antibodies respectively, according to classical techniques [4]. The distribution has been studied in 31 controls and 22 primary MGN.

Results

HLA typing

The distribution between controls and primary MGN is not different except for two antigens, B8 and DR3. The B8 frequency is 14.42 per cent in controls compared with 57.14 per cent in primary MGN (Pc=0.00018). The DR3 frequency is 20.27 per cent in controls and 65.38 per cent in primary MGN (Pc=0.00008). The B8 DR3 association frequency is 10.81 per cent in controls versus 50 per cent in MGN patients. The relative risk of MGN is between seven to eight for subjects bearing either the B8 or the DR3 antigen.

Sensitised erythrocytes half-life

In nine volunteers, the half-life is 35.36 ± 8.50min compared with 1752min in two splenectomised and 12min in a patient with hypersplenism. In 18 primary MGN patients the half-life is prolonged to 67.05 ± 69.64min. However, the distribution showed that only one-third of patients had significantly prolonged values over the normal range.

Blood T-lymphocyte subpopulations

The respective values in controls and MGN are as follows: 63.77 ± 0.37 per cent versus 53.74 ± 13.31 for OKT3-positive cells; 37.90 ± 8.21 versus 32.79 ± 10.89 for OKT4 positive cells; 21.60 ± 5.28 versus 20.03 ± 5.76 for OKT8-positive cells; and 1.85 ± 0.58 versus 1.74 ± 0.69 for T4/T8 ratio. The decrease in OKT3 and OKT4 fractions in MGN is significant ($p<0.05$).

Discussion

We confirm that primary MGN is strongly associated with HLA-B8 or/and HLA-DR3 antigens.

The clearance of autologous labelled and IgG sensitised erythrocytes is a satisfactory parameter of Fc receptor-mediated phagocytosis by splenic macrophages. In MGN patients, the clearance is totally defective in one-third of the patients. This abnormality is not constant, but almost always presents in B8 or/and DR3 positive MGN patients during the acute phase. It could therefore be genetically controlled.

The blood T-lymphocyte subpopulations show a decreased helper T-cell fraction in the MGN group. However this finding correlated with patients in complete remission. During exacerbation the T4/T8 ratio tends to be high (2.00 ± 1.02) with a significant decrease during remission (1.43 ± 0.54), and an intermediate value during the chronic phase (1.77 ± 0.33). These data and others [5] suggest hyperactivity of helper T-cells during exacerbations, which may be secondary to a functional deficiency of suppressor T-cells bearing IgG-Fc receptors.

These data support the following hypothesis, two factors are needed: one acquired acting as a trigger and corresponding to the introduction of T-dependent antigens, and one genetic with a latent, subtle immunodeficiency. This deficiency could be a cellular IgG-Fc receptor dysfunction both on suppressor T-cells and on fixed and circulating macrophages.

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References

1 Klouda PT, Acheson EJ, Goldby FS et al. *Lancet 1979; ii:* 770
2 Lawley TJ, Hall RP, Fanci AS et al. *N Engl J Med 1981; 304:* 185
3 Frank MM, Hamburger MI, Lawley TJ et al. *N Engl J Med 1979; 300:* 518
4 Reinherg EL, Schlossman SF. *Cell 1980; 19:* 821
5 Chatenoud L, Bach MA, Jungers P et al. *Immunology Letters 1980; 2:* 167

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ZUCCHELLI (Italy) We have the same results in T4/T8 ratio. During the active phase of the nephrotic syndrome we have high ratio and during resolution of the nephrotic syndrome the ratio becomes normal. Individual values obtained different results, so we have two different groups of patients, approximately fifty per cent with a high ratio and approximately fifty per cent with normal ratio. Do you have any data about individual ratio during the active phase and the prognosis on the evolution of the patients?

BERTHOUX No, we are now trying to follow patients first in an exacerbation then three months after, and one year after. We are trying to study the same patient during exacerbation phase, in the chronic phase and we hope in remission. At present we have very few patients.

VAN DER WOUDE (Groningen) Did you always use the same red cells for the antibody count?

BERTHOUX The red cells are coming from the patient, autologous red blood cells, and it can only be done in rhesus positive patients.

VAN DER WOUDE How do you show that your antibody coating is always the same because the rhesus phenotype will differ between the patients. The antibody coating is of crucial importance for the half-life time. As you know with radiolabelled antiglobulin preparations a difference in antibody coating is of crucial importance for the $T_{1/2}$.

BERTHOUX We checked the full rhesus phenotype but this does not change and in fact the technique is very reproducible. Some of our colleagues did some similar studies on other patients using their own controls and had exactly the same values.

VAN DER WOUDE That is not in agreement with our fairly extensive studies.

BERTHOUX We have always used the same batch of pure anti-D IgG and it is very reproducible.

VAN DER WOUDE The second question is do you really get monoexponential curves because in a recent paper in The Journal of Rheumatology a Canadian group described a very clear cut bi-exponential curve during this experiment and again we can confirm this. We did not get monoexponential curves and as you are speaking about $T_{1/2}$ time I think this should only be done when there is a monoexponential curve.

BERTHOUX That is a general comment with kinetics and nuclear tracers. If you waited one week, two weeks you can just get one, two, three or four exponentials.

VAN DER WOUDE That is not true, because when you look during one hour you get two very distinct curves.

BERTHOUX No, I disagree with you. We perform our study over sixty minutes at five points, 10, 20, 40, 50 and 60 minutes, and we obtain a straight line.

VAN DER WOUDE How sure are you that everything goes to the spleen? We showed that in membranous glomerulopathy the liver component of RES function increased significantly.

BERTHOUX We did some camera studies to be sure.

MALLICK (Manchester) Following our earlier report to which you referred I would like to ask if you have any evidence on the factor B allele F1. We were able to show that not only is DR3 connected but BFF1 is also on the same chromosome strongly linked as has been shown for juvenile diabetes. We found this very strongly associated with membranous nephropathy and have a suggestion that it may be associated with poor prognosis.

Did you have DR3 controls for your IgG labelled cells? Clarkson from Australia has done this and shown that compared with DR3 negative controls DR3 positive controls have a prolonged half-life. In four studies, one of which has been published, the evidence seems to be that half the patients with membranous and DR3 have abnormalities in half-life and half do not and I do not think myself that the association is with membranous, I think it is with DR3.

BERTHOUX I am aware of your work, we are just now doing Bf allotypes and also C2, but we have no results. For your second question, of course we were very upset about this data. In our control we have only three patients who are B8 DR3 positive, but they all have normal half-lives, so may be we have to go back and get more normal volunteers who are B8 DR3 positive. If we analyse all our MGN group, of course we have no correlation between B8 DR3 and prolonged half-life because I think it depends on the stage of the disease. During exacerbation all, except one in our series, the B8 DR3 positive patient, have a prolonged half-life. I think it is not a permanent defect, it is a latent defect expressed only during the acute phase.

MATOUŠOVIC (Prague) Did you study the changes of the T-cell subpopulation after corticosteroid therapy. We studied suppressor T-cell activity after concanavalin A stimulation in vitro. We found the increase in this activity during methylprednisolone treatment in some patients with membranoproliferative glomerulonephritis, but in other ones we did not. We can't still explain this finding, but suppressor T-cell activity was constantly decreased during the relapse of minimal change nephrotic syndrome and increased significantly after methylprednisolone treatment when proteinuria disappeared. This increase persisted for several weeks, but before a relapse it decreased again.

BERTHOUX I agree with you. It might be a clearance of steroids having an effect on the T4/T8 ratio. None of our patients were on steroids during the study. Patients in exacerbation were studied before treatment and for patients in remission some had previously had steroids but they were not on steroids at the time of the assay. We are trying to have longitudinal studies in the same patients starting acutely so we get the T4/T8 ratio before any treatment, and after three months treatment with corticosteroids. At present we have just a few patients so we cannot comment.

MATOUŠOVIC But the changes in suppressor T-cell activity after corticosteroids persist for many weeks. It is also affected after treatment with Cyclophosphamide.

BERTHOUX Yes, it could be, but some patients were in a spontaneous remission.