EPSTEIN-BARR VIRUS SPECIFIC ANTIBODIES IN PATIENTS WITH IgA NEPHROPATHY

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SYNOPSIS

Serum EBV-specific antibodies of both the IgG and IgA classes were measured in 58 patients with IgA nephropathy (IgAN), 39 patients with non-IgA glomerulonephritis, 41 patients with nasopharyngeal carcinoma (NPC) and 36 normal subjects. Detection of these antibodies was achieved by the immunoperoxidase staining technique using the Jiyoye cell line which manifests the EBV viral capsid antigen (VCA).
IgG-EBV antibodies were detected in at least 89% of patients in all study groups indicating that exposure to the EB virus is very common among the local population. Serum EBV-specific IgA antibodies were not significantly raised in patients with IgAN.
83% of NPC patients had high titres of serum IgA-EBV antibodies of the A1 subclass. 7% of these patients were albuminuric and it remains uncertain if they have underlying IgA nephropathy as histologic diagnosis was not made.
It is concluded that EB virus infection is unlikely to play a direct role in the pathogenesis of IgA nephropathy. The high incidence of elevated serum IgA-EBV antibodies in NPC patients is confirmed.

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INTRODUCTION

IgA nephropathy (IgAN) was first described in 1968 by Berger and Hinglais(1). Histopathologically it is characterised by proliferation of mesangial cells with predominant mesangial IgA deposits in the mesangium. Clinically, it's main features are haematuria, proteinuria and elevated serum IgA levels in a large number of cases(11). The disease manifested by gross haematuria is often exacerbated by upper respiratory or gastrointestinal infection with temporal rise in serum antibody to specific infective agent. These infections may be of viral origin and it has been proposed that IgA nephropathy may be related pathogenetically to a persistent viral infection in the tonsils(2,3).

Epstein-Barr virus (EBV) is an ubiquitous human herpesvirus as judged by a high frequency of seropositivity in adult populations throughout the world(4,5). It is the cause of infectious mononucleosis(6) and possibly of nasopharyngeal carcinoma (NPC)(7) and Burkitt's lymphoma(8). High levels of EBV-specific IgA antibodies in the serum had been reported as an outstanding feature of patients with NPC(9). It is not known if NPC patients have a high incidence of IgAN. We wondered if serum EBV-specific IgA antibodies were also present in higher titres in patients with IgAN.

The aim of this study was to measure serum EBV-specific IgA antibodies and to determine their subclasses in IgAN patients, non-IgAN glomerulonephritis (GN) patients, NPC patients and normal subjects.

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MATERIALS AND METHODS

Subjects

Subjects included in the study were 58 patients (12 males, 24 females; mean age 30+/-11 years) with IgAN, 39 patients (32 males, 26 females; mean age 29+/-9 years) with non-IgA glomerulonephritis, 41 patients (26 males, 13 females; mean age 28+/-11 years) with NPC and 36 normal subjects (all adults). Patients with systemic lupus erythematosis, Henoch-Scholein Purpura and chronic liver diseases were excluded.

Detection Of Antibody To Epstein-Barr Virus

a. EBV-VCA Source

The Jiyoye cell line expressing EBV viral capsid antigen was used to make cell spreads. The cell line was maintained in RPMI 1640 with glutamine, with 20mM Hepes (Flow Lab) and supplemented with 2 g/l sodium bicarbonate, 125 I.U./ml penicillin, 125 ug/ml streptomycin sulphate and 10% foetal calf serum. To make cell spread, cells were washed 3 times with phosphate buffered saline (PBS) and resuspended at a concentration of 3x10(6) cells per ml. One drop of this cell suspension was put onto each well on a 8- well coated glass slide. The cell spreads were dried on a slide drying plate for 30 minutes, cooled and then fixed for 10 minutes in cold acetone at 4oC. Slides were stored at -80oC until used.

b. IgG and IgA Antibodies

The immunoperoxidase staining technique was used to detect EBV- specific IgG and IgA antibodies. The staining procedure was as follows:

All washings were done in PBS bath on magnetic stirrer.

1. Frozen slide containing Jiyoye cell spread was thawed at room temperature and then washed for 5 minutes.

2. 50 ul of a 10% normal rabbit serum (NRS) was applied and incubated for 30 minutes to block non-specific protein binding. Rinsed briefly.

3. 50 ul of test serum at appropriate dilution (diluent: 1% BSA, 0.01% sodium azide in PBS) was then applied and incubated for 30 minutes.

4. Slide was washed thoroughly for 10 minutes with 3 changes of PBS bath.

5. 50 ul of peroxidase conjugated rabbit antibody to human IgG or IgA (Dako, Copenhagen, Denmark) was applied at 1:40 dilution (diluent: 1% FCS, 1% NRS, 0.01% sod. azide in PBS) for 30 minutes.

6. Slide was washed thoroughly for 10 minutes with 3 changes of PBS bath.

7. Freshly prepared and filtered substrate solution (6 mg 3,3 diaminobenzidine tetrahydrochloride dissolved in 10 ml of 0.05 M Tris buffer, pH 7.6 plus 10 ul of 30% hydrogen peroxide) was applied for 5 minutes to develop brown staining. The slide was washed under tap for 5 minutes.

8. The slide was then counterstained by dipping briefly in filtered haematoxylin and washed under running tap for 5 minutes.

9. Next the slide was dehydrated in alcohol gradient (free of eosin) and cleared in xylene.

10. It was finally dried in air, coverslipped and mounted in DPX mountant for examination using a light microscope.

c. Sub-Typing IgA Antibodies

Test sera found to be positive for EBV-specific IgA antibodies were sub-typed for IgA1 and IgA2 subclasses. Mouse monoclonal antibody to human IgA1 or IgA2 (Becton Dickinson) at 1:10 dilution and peroxidase conjugated rabbit antibody to mouse immunoglobulins at 1:10 dilution were sequentially applied after the test serum. The rest of the staining procedure was as described above.

URINE ALBUMIN/URINE CREATININE RATIO

Urine albumin was measured by radial immunodiffusion using rabbit antibody to human albumin (Behring). Urine creatinine was measured by the alkaline picrate method.

RESULTS

Antibody To EBV-VCA

EBV-specific IgG antibodies were measured at 1:10 dilution of test sera. Among the 10 normals, 9 IgAN, 9 non-IgAGN and 9 NPC patients studied, the percentage positivities obtained were 89% and above (Table I). Titering was done only at the 1:80 dilution level. At this dilution level, percentage positivity remained at 100% for NPC patients but fell to 56-78% in the other 3 studied groups indicating that higher titers were found in NPC patients.

EBV-specific IgA antibodies were measured at 1:5 dilution of test sera (Table II). In 58 patients with IgAN, 17% were positive. This percentage was not significantly higher than the 11% measured in 36 normal subjects and it was very close to the 18% found in 39 patients with non-IgA GN. As reported in the literature most NPC patients were positive, 83% in the present series of 41 patients.

Titering was done only on positive cases. The geometric mean titre (GMT) was essentially the same for the normal, IgAN and non-IgA groups at 5,6 and 6 respectively. Only among the 12 NPC patients evaluated was the GMT raised very significantly to 58.

TABLE I
EBV-SPECIFIC IgG ANTIBODIES

	% Positive (at 1:10)	% with Titre 80
Normal (10)	100	70
IgAN (9)	100	56
non-IgA (9)	89	78
NPC (9)	100	100

TABLE II
EBV-SPECIFIC IgG ANTIBODIES

	% Positive (at 1:5)	GMT (positive cases)
Normal (36)	11	5 (4)
IgAN (58)	17	6 (10)
non-IgA (39)	18	6 (7)
NPC (41)	83*	58* (12)

GMT – geometric mean titre
*$p < 0.001$

Sub-typing Of EBV-Specific IgA Antibodies

The determination of IgA subclasses was attempted with the positive cases. This was not successful with the normal, IgAN and non-IgA GN groups (Table III) probably because of the very low titers of IgA antibodies. Among the 11 NPC patients studied, 5 were positive for IgA1 but none were positive for IgA2.

NPC PATIENTS WITH/WITHOUT ALBUMINURIA

Renal involvement in patients with NPC was assessed by measuring the urine albumin/urine creatinine ratio. In 50 normal subjects examined, the ratio was .025 at 95% confidence limit. Among 90 patients with NPC, 6 had a ratio 0.025 indicating some renal involvement. These 6 patients were compared with another 6 NPC patients who had no albuminuria. As summarized in Table IV, apart from the

TABLE III
SUB-TYPING OF EBV–SPECIFIC IgA ANTIBODIES

	IgA1	IgA2
Normal (4)	0	0
IgAN (8)	0	0
non-IgA (5)	0	0
NPC (11)	5	0

absence or presence of albuminuria, there was no significant difference between the two groups with respect to EBV-IgA titres, presence of IgA1 and absence of IgA2.

DISCUSSION

Serum EBV-specific IgG antibodies were demonstrated in 89% and above in the three patient groups and in normal subjects. This is in agreement with a recent report(12) which showed same frequency of serum IgG antibodies to EBV-viral capsid antigen (VCA) in IgAN patients and normal controls. This indicates that exposure to EBV is very common among the local population, and confirms reports of its ubiquitous occurence in other parts of the world(5).

Serum EBV-specific IgA antibodies were not found to be raised or more frequent in our patients with IgAN suggesting that EBV infection may not play a direct role in its pathogenesis. Our results differ from a recent finding(12) of significantly more frequent occurence of IgA antibodies to EBV-VCA in IgAN patients. The reason(s) for the conflicting observations is unknown. It is currently not possible to detect the EBV-related antigens in renal tissue as specific antisera are not commercially available. Previous workers(13) have also demonstrated raised EBV antibody titres in various forms of GN excluding IgAN. Different types of GN have also been associated with EBV mononucleosis(14,15).

The association between viral infection and IgAN was demonstrated by Tomino et al(10) who passaged virus(es) by coculturing Vero cells (renal fibroblasts of African green monkey) with freezed- thawed extracts of tonsillar cells from patients with IgAN. They were then able to demonstrate binding to the nuclear region of these cells by 125 I-labelled eluate of mesangial IgA antibodies from the same or other patients with IgAN.

The high levels of EBV-specific IgA antibodies in patients with NPC was confirmed. The percentage positive was 83% and the geometric mean titre was 58. The IgA subclass was A1. Renal involvement among 90 NPC patients investigated by urine albumin/urine creatinine ratio was 7%. It is not known if these 6 patients have IgA nephropathy without renal biopsy being done. Further study to investigate the EBV-associated antigens in renal tissues of these patients should be helpful in our efforts to elucidate the etiology and pathogenesis of IgA nephropathy.

In conclusion, our findings indicate that EB virus infection is unlikely to play a direct role in the pathogenesis of IgA nephropathy. The high incidence of elevated serum IgA-EBV antibodies in NPC patients is confirmed.

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TABLE IV
COMPARISON OF NPC PATIENTS WITH/WITHOUT ALBUMINURIA

	+ Albuminuria	– Albuminuria	p
u. ALB/u. Cr	0.130–1.228	< 0.025	< 0.001
EBV — IgA (GMT)	60	56	ns
EBV — IgA1	2/5	3/6	ns
EBV — IgA2	0/5	0/6	ns

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