

Specific IgE detected by ELISA and immunoblot after human cytomegalovirus infection (HCMV) in renal transplant (RT) recipients.

Manuel A. Vargas^{a,*}, Francoise Bertrand^b, Kipanga N. Mulongo^a, Jean P. Squifflet^c,
Monique E. Lamy^a

^aDepartment of Virology, Catholic University of Louvain, 30/55 Clos Chapelle aux Champs, 1200 Brussels, Belgium

^bDepartment of Epidemiology, Catholic University of Louvain, 1200 Brussels, Belgium

^cDepartment of Surgery, Catholic University of Louvain, 1200 Brussels, Belgium

Received 23 September 1995; accepted 21 January 1996

Abstract

Background: Specific HCMV IgE response has been reported by some authors, and was proposed as a valuable virologic marker of CMV infection.

Objectives: we evaluated specific HCMV IgE in renal transplant patients with active (primary and secondary) HCMV infection with special interest to symptomatic infections.

Study design: Specific IgE was tested retrospectively by ELISA and immunoblot (IB) on sera of 55 RT patients who were followed before and after transplantation with virologic markers of CMV infection.

Results: Total serum IgE levels were similar in control group and in patients with primary and secondary HCMV infections. Anti-CMV specific IgE response by ELISA was more frequently found in patients with primary infection (76.9%) than in patients with secondary infection (47.1%). These specific IgE reacted on immunoblot with a 150 kDa protein in 84.6% of patients with primary infection and 94.1% with secondary infections; and reacted with rp52 (pUL44) in 76.9% of primary infection and 47.1% of secondary infection.

Conclusions: Anti-CMV specific IgE tested by immunoblot and ELISA is a marker of CMV infection. It was clearly detected in cases of active infection (primary and secondary) and was present in cases with severe CMV clinical manifestations. In contrast, anti-CMV specific IgE, was consistently negative among healthy blood donors. This is the first report of CMV proteins detected by IgE immunoblot.

Keywords: Human cytomegalovirus; Renal transplant recipient; Diagnosis; IgE response; Immunoblot

1. Introduction

Abbreviations: HCMV, Human cytomegalovirus; RT, Renal transplant patients; IB, Immunoblotting.

* Corresponding author. Tel.: + 32 2 7643420; fax: + 32 2 7643163.

Increased total serum IgE titers were described in the acute phase of respiratory tract infections (Perelmutter et al., 1979), active periods of fre-

quent herpes simplex recurrences (Lagacé-Simard et al., 1986) and in patients with HIV infection (Wright et al., 1990; Israël-Biet et al., 1992; Fressard et al., 1994). Specific IgE responses were detected in severe lower respiratory diseases caused by respiratory syncytial virus (RSV); higher titers of RSV-IgE were found during convalescence (Welliver et al., 1981). Parainfluenza-virus IgE specific titers were correlated with the presence of severe illness (Welliver et al., 1982). Specific HCMV-IgE response was described by some groups (Van Loon et al., 1985; Nielsen et al., 1988; Weber et al., 1992, 1993). The aim of this retrospective study was first to compare specific IgE with other markers of active CMV infection; secondly to establish a correlation between specific CMV IgE response and clinical manifestations, and finally to define the HCMV proteins recognized by IgE.

2. Materials and methods

2.1. Patients

A group of 55 RT patients with virologic data for CMV infection was studied retrospectively. The basic inclusion criterion was the availability of sera during the whole follow-up period starting before transplantation and going up to 300 days after transplantation. CMV serology was tested before transplantation in each donor and recipient pair. Thirty seven men with a mean age of 36.2 years (range 4–57 years) and 18 women with a mean age of 33.7 years (range 7–57 years) entered the study.

As induction immunosuppressive regimen all transplant recipients received: a combination of rabbit antithymocyte globulins (RATG, Frese-nius, Germany) for the first 10 days, associated with azathioprine (Imuran, Wellcome) 1 mg/kg/day, cyclosporin A (Sandimmun, Sandoz) starting at 3 mg/kg/day and then progressively increased up to 5–10 mg/kg/day (according to cyclosporine and creatinine levels) and prednisolone, starting at a dose of 0.5 mg/kg/day and progressively decreased to 0.1 mg/kg/day. Rejection episodes were treated with a 10-day course of

5 mg/day OKT3 (Orthoclone, Cilag, Switzerland). None of the patients received hyperimmune anti-CMV globulin as prophylaxis during the period of the study.

2.2. Sera

A total of 246 sera from patients with primary and secondary infections was tested in parallel for IgM, IgG, and CF CMV antibodies; from these sera 137 specimens selected around the period of active infection were tested for specific IgE response.

The detection of CMV infection was performed by antigenemia, culture and serological tests as described previously (van der Bij et al., 1988; van den Berg et al., 1989).

2.3. ELISA procedure to test IgE CMV antibodies

The ELISA IgE is an antibody capture assay. Individual plates (Maxisorp U16 Nunc) were coated with 50 μ l of a mixture of rat monoclonal antibodies, LOHE-10 and LOHE-17 (Bazin, 1981) kindly provided by Prof. H. Bazin, Catholic University of Louvain, each one at a concentration of 5 μ g/ml in 0.05 M carbonate bicarbonate buffer pH 9.6 and kept overnight at 4°C. Afterwards, plates were washed three times with PBS 0.1% Tween 20. Plates were then saturated with a 5% gelatine solution in PBS 0.1% Tween 20 for 2 h at 37°C. The plates were washed again, as before, and incubated at 4°C overnight with patients' sera at 1/40 dilution. The next day plates were washed three times and then incubated for 2 h at 37°C with 50 μ l of peroxidase labeled CMV antigen (Medac, Hamburg), after which they were washed again. Then 50 μ l of substrate solution (orthophenyldiamine 10 mg in 5 ml of citrate buffer, H₂O 2 30% 1 μ l pH 5) was added to each well; after 10 min in the dark at room temperature, the reaction was stopped with 0.1 ml of H₂SO₄ 1N. Finally, within the following 10 min, absorbance was read by photometry at 492 nm and 650 nm as reference wavelength. All tests were performed with a single lot of monoclonal antibodies and CMV antigen. Results were ex-

pressed in a standardized way to take into account the daily variation of the optical density measurement. The standardized absorbance (A_{st}) was defined as the observed optical density (D_o) of the sera minus the mean daily absorbance (m) divided by the daily standard deviation (S.D.) of 5 negative controls ($A_{st} = D_o - m / \text{S.D.}$). Positive values are those greater than 3. Negative controls sera were selected from a group of 20 healthy CMV IgG seropositive blood donors.

2.4. Immunoblotting

The IgM, IgG and IgE responses were detected by immunoblot (IB) using purified virions as described previously (Landini et al., 1985). IgM and IgE reactions were additionally tested by IB using recombinant non-structural protein rp52 or pUL44 (Ripalti et al., 1994). The separated proteins were then electrophoretically transferred to nitrocellulose membranes (BA 85 0.45 μM , LKB, Sweden) by a semi-dry electrophoretic blotter. Residual binding capacity was blocked by incubating immunoblot for 60 min with Tris-NaCl (TBS, 20 mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween 20) containing 0.5% casein. Sera for IgM and IgE detection were treated by adding 0.2 ml of RF absorbent (resuspended in 1.5 cm^3 of blocking solution, Behringwerke, Marburg, Germany) to 0.2 ml of serum to eliminate the competitive action of IgG and thereafter diluted 1:4. Horseradish peroxidase-coupled rabbit antihuman IgM and IgE (DAKO, Denmark) were used respectively at 1:400 and 1:125 dilution. For IgG immunoblot untreated sera at 1:32 dilution was used. A horseradish peroxidase-coupled rabbit anti-human IgG (DAKO, Denmark) was used at 1:400 dilution.

2.5. IgE levels

IgE level was measured by a latex agglutination test. Titres are expressed in IU/ml by reference to a standard IgE preparation.

2.6. Clinical score test system

The severity of CMV disease was evaluated using a score test system as suggested by Plotkin et al. (Ljungman and Griffiths, 1993).

Primary infection was defined as seroconversion (IgM, IgG and/or CF test) for CMV with or without CMV isolation, with or without positive antigenemia test.

Secondary infection was defined as a fourfold rise in CMV antibody titers in previously seropositive patients with or without CMV isolation, with or without positive antigenemia test.

2.7. Statistical analysis

Two sided *t*-tests and variance analysis were used. The significance level was set at 0.05.

3. Results

Of the 55 RT recipients, 30 were seronegative before transplantation. Thirteen of them seroconverted, with 12 (92.3%) receiving a kidney from a seropositive donor. Out of the remaining 25 patients who were seropositive before transplantation, 17 experienced a secondary infection, with 13 of them (76.5%) receiving a kidney from a seropositive donor. Results of virologic markers from patients with primary and secondary infections are presented in Table 1.

3.1. Total IgE

The mean total serum IgE level was 27.25 IU/ml in CMV seropositive blood donors. The mean total serum IgE level in the group of patients with a primary infection was 30.69 and in the group with a secondary infection was 20.77.

3.2. Specific IgE

In the group of primary and secondary infections the average of maximum standardized absorbance of specific CMV IgE is 9.32 and 2.69, respectively. Patients with primary infection and pneumonia presented maximum CMV IgE stan-

Table 1

Virologic markers of active HCMV infection in two groups of renal transplant recipients

	Primary		Secondary	
	(+ /No. tested)	(%)	(+ /No. tested)	(%)
Antigenemia	10/12	83.3	12/16	75
Culture				
Hemoculture	3/12	25	2/17	11.8
Bronchoalveolar lavage	2/2	100	0/1	0
Urine	12/13	92.3	16/17	94.1
Saliva	5/10	50	9/17	52.9
IgM ELISA	13/13	100	9/17	52.9
IgM Blot virion	13/13	100	17/17	100
IgM Blot p52	13/13	100	14/17	82.4
IgE ELISA	10/13	76.9	8/17	47.1
IgE Blot virion	12/13	92.3	16/17	94.1
IgE Blot p52	10/13	76.9	8/17	47.1
IgG ELISA ^a	13/13	100	17/17	100
IgG Blot virion	13/13	100	17/17	100
CF Test ^a	13/13	100	16/17	94.1 ^b

^aFour-fold increased in titer.^bOne case with high CF titers previous to recurrency.

dardized absorbance with a mean of 18.44, while those without pneumonia showed a CMV IgE mean standardized absorbance of 3.70. The 17 patients who remained seronegative and the 8 patients who remained seropositive without a secondary infection after transplantation showed a mean standardized absorbance similar to that of healthy blood donors control group. In this group of healthy donors, the optical density (OD) values were less than 0.289 and the standardized absorbances (St Ab) were less than 3

3.3. CMV IgM immunoblot

Sera from primary infection presented positive IgM IB results in 100% of cases. These sera from primary infection reacted by IgM IB with a mean of 7.5 proteins (range 2–12). The 65 kDa and 38 kDa polypeptides were detected in all cases, whereas other polypeptides with a high incidence of positive reactions were 150 kDa (84.6%), 116 kDa (76.9%), 60 kDa (76.9%), 55 kDa (76.9%) and 72 kDa (69.2%). IgM reaction to rp52 was found in all cases of primary infection. For secondary infections IgM reaction was detected by

IB in 100% of cases. Sera from secondary infection reacted with a mean of 4.6 proteins (range 2–9). The 38 kDa polypeptide was detected in 94.1% of cases, while other polypeptides with a high incidence of positive reactions were 150 kDa (70.6%), 55 kDa (64.7%), 65 kDa (52.9%), 60 kDa (47.1%), 28 kDa (47.1%) and 116 kDa (35.3%) and 85 kDa (35.3%). IgM reaction to rp52 was found in 82.4% of cases with secondary infection.

3.4. CMV IgG immunoblot

Positive IgG reactions were detected by IB in 100% of cases with primary and secondary infections. Sera from primary infection reacted by IgG IB with a mean of 3.5 proteins (range 1–8). The 65 kDa polypeptide was detected in 84.6% of sera from primary infection, whereas other polypeptides with a high incidence of positive reactions had molecular weights of 55 kDa (69.2%), 28 kDa (53.8%), 150 kDa (30.8%), 85 kDa (30.8%) and 38 kDa (30.8%). In cases of secondary infection sera reacted with a mean of 7.8 proteins (range 3–10). The 65 kDa polypeptide was detected in all cases of secondary infection, while other polypeptides

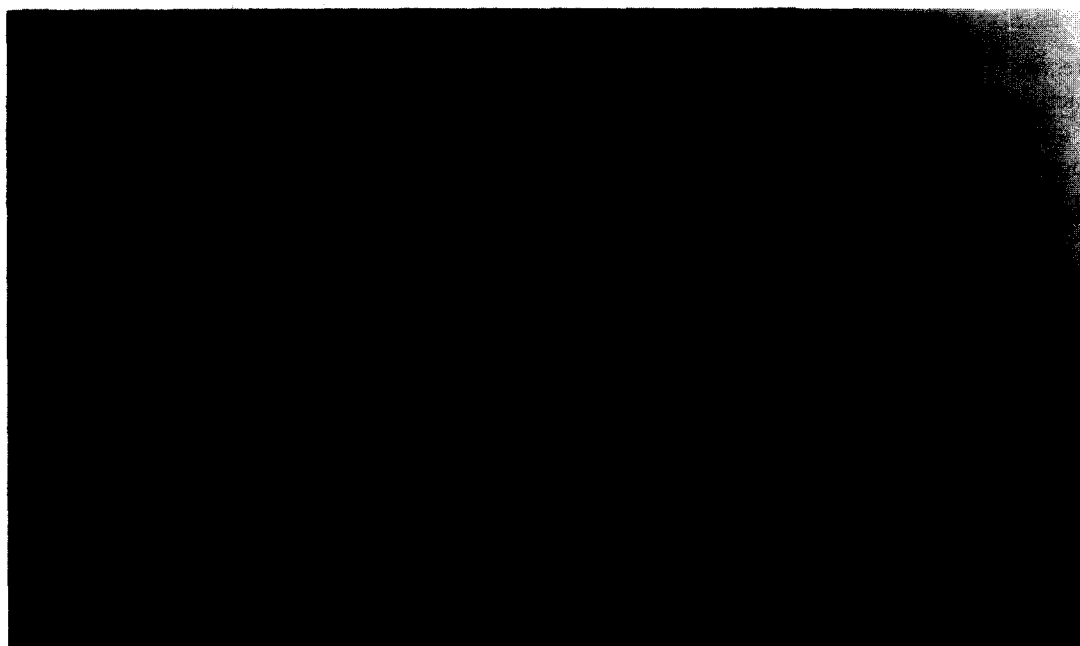


Fig. 1. Specific IgE response to rp52 in sera from patients with human cytomegalovirus primary infection. Lanes 1–5: cases with pneumonia; lanes 6–13: primary infections; lane 13–15: IgE controls.

with a high incidence of positive reactions had molecular weights of 67 kDa (94.1%), 55 kDa (94.1%), 150 kDa (82.4%), 96 kDa (82.4%), 38 kDa (82.4%), 28 kDa (82.4%) and 45 kDa (64.7%).

3.5. CMV IgE immunoblot

Immunoblot showed that IgE response was mostly directed to a protein of 150 kDa in primary infection (84.6%) and in secondary infection (94.1%). In primary infection IgE reacted with 28 kDa in 38.5% of cases and with 55/58 kDa in 23.1% of cases. In secondary infection 35.3% of serum reacted with 55/58 kDa and 28 kDa. IgE reaction to rp52 was found in 76.9% of primary infection (Fig. 1) and 47.1% of secondary infection.

3.6. Clinical manifestations

As shown in Table 2, clinical manifestations were present in 13 out of 13 patients (100%) with primary infection, and in 7 out of 17 patients

(41.2%) with secondary infection. In primary infection clinical manifestations were found with a mean score test of 4.54; in secondary infection the mean score test was of 0.82. Cases of primary infection with pneumonia had a mean score test of 7 and cases of primary infection without pneumonia had a score test of 3.

Table 2
Clinical manifestations during the course of 13 primary and 17 secondary infections

	Primary		Secondary	
	(+)	(%)	(+)	(%)
Fever	12	92.3	3	17.6
Leukopenia	10	76.9	5	29.4
Thrombocytopenia	4	30.8	2	11.8
Liver enzymes	4	30.8	1	5.9
Pneumonia	5	38.5	1	5.9
Superinfection	3	23.1	0	0
Renal insufficiency	2	15.4	0	0
Jaundice	1	7.7	0	0
Arthritis	1	7.7	1	5.9

4. Discussion

There are numerous virologic markers for CMV active infection. However, very few of them detect symptomatic infections. In that respect, antigenemia (van der Bij et al., 1988; van den Berg et al., 1989; Lamy et al., 1994), together with PCR (Zipeto et al., 1993) is currently considered as the most accurate test. Other virologic markers are frequently present in primary and secondary infections, but they detect the infection with such a delay (3–32 days) that they do not allow rapid therapy.

In our series CMV specific IgE response was found more frequently in cases of primary infection (76.9%) compared to cases of secondary infection (47.1%). The maximal standardized absorbance had a tendency to be higher in the group of primary infection compared to the group of secondary infection ($P = 0.057$). This is in line with previously reported articles. Within the primary infection group the values of specific IgE standardized absorbances had a tendency to be higher in those patients presenting pneumonia ($P = 0.08$). We confirmed these observations by using the clinical score test system. We found a significantly higher score test in the primary infection group compared to the secondary infection group ($P = 0.0001$). Furthermore all the cases with primary infection and pneumonia had a higher score test than those without pneumonia ($P = 0.0001$). In cases of primary infection with pneumonia in which total serum IgE and specific CMV IgE were measured in parallel, we found that their kinetics was independent from each other (Fig. 2); which gives support for the specificity of anti-CMV IgE response and shows that specific CMV IgE are not the result of an increased total serum IgE level. In our series total serum IgE was similar in control group and in patients with primary and secondary infections (difference not significant, $P = 0.63$, ANOVA test). Thus increased specific IgE could be a result of T cell deregulation in the IgE synthesis induced either directly by the virus or indirectly by regulators of IgE production like IL-4, IL6, IFN (Vercelli and Geha, 1991; Geha, 1992).

However, some authors (Weber et al., 1992, 1993) found the incidence of specific IgE to be equivalent in primary and secondary infections. These discrepancies could be attributed to differences in tests sensitivities, in antigen used for IgE detection or in the level of immunosuppression of the studied cases, and therefore in the upregulation of IgE production in these series.

Many reports were published concerning CMV IgG and IgM reponse by immunoblot (for a review see Landini, 1992). In our study IgM and IgG reacted with an important number of viral proteins (Table 3).

Our IgE immunoblot reacted less and with fewer proteins than the immunoblot of IgG and IgM. Comparing reactivities of immunoblot with purified virions and recombinant protein p52 we found that IgE response was stronger with the recombinant protein. This could be due to a higher concentration of recombinant protein or to a higher affinity of CMV-IgE to rp52. In our hands specific IgE IB proved to be a sensitive tool for CMV infection diagnosis: it detected all cases of primary infection in agreement with ELISA and more cases of secondary infection than Elisa. However four cases negative for ELISA reacted with a 150 Kda protein; these cases consisted of one symptomatic primary infection (score test 2) and three secondary infections, two of them being symptomatic (score test 1); the question of false positive results must be raised. (Lazzarotto et al., 1992) studying IgM response found this type of reaction in false positive sera and attributed the reaction to the major capsid protein p150 (pUL86) that has some cross-reacting regions with herpes viruses. In IgE this finding needs to be further clarified. Using rp52 all cases of primary-infection positive by specific

Elisa IgE were confirmed by immunoblot. In secondary infections four cases which were negative by ELISA IgE gave positive reactions by immunoblot. This first report on CMV proteins detected by IgE immunoblot shows the usefulness of this marker to confirm diagnosis of CMV infection. Furthermore, it could help to study CMV IgE response in different clinical conditions and thus the role of IgE in the pathogeny of CMV infection.

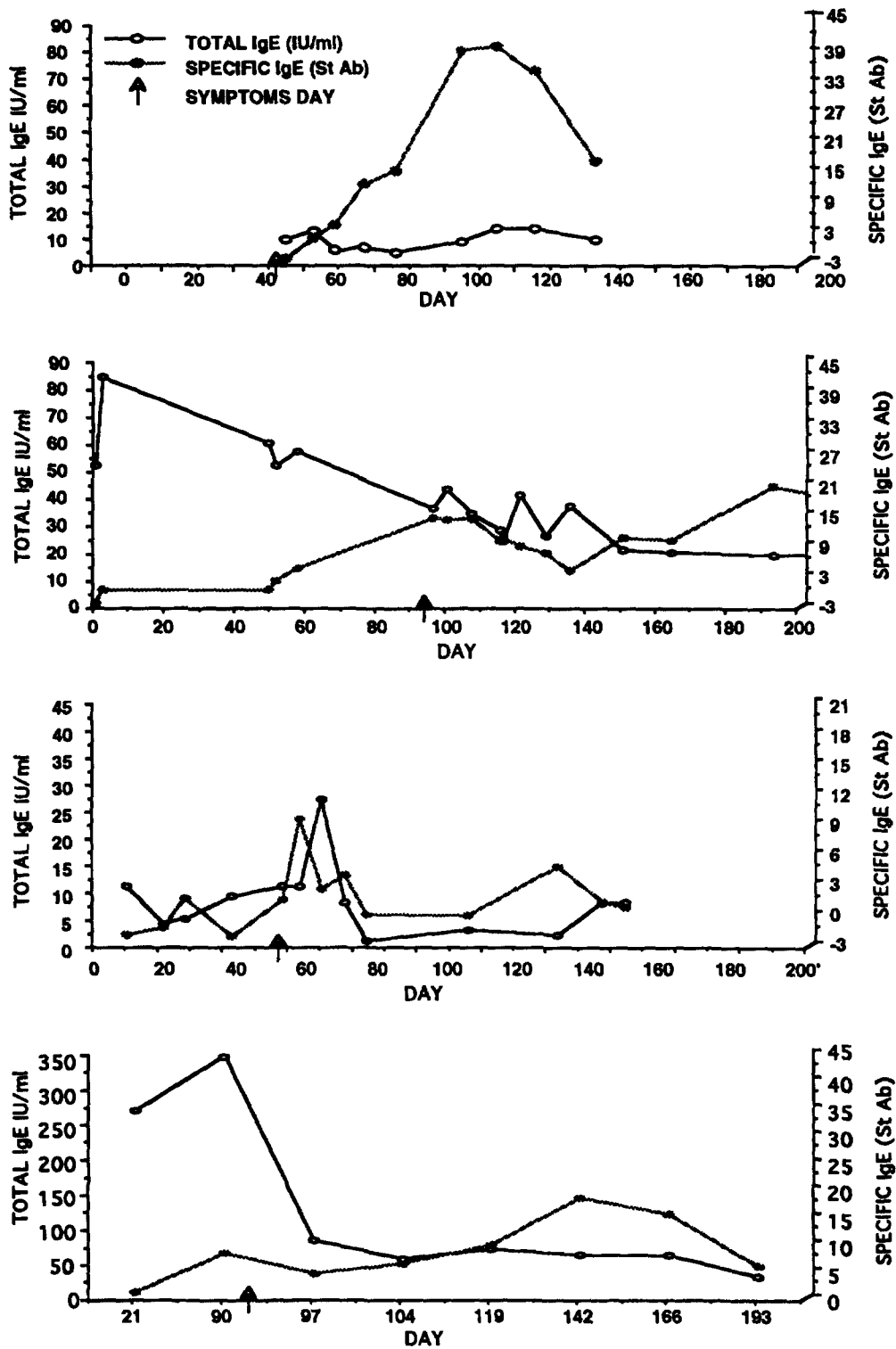


Fig. 2. Day 0 is the day of transplantation.

Table 3
Percentage of proteins detected by blot in two types of CMV infection

Protein kDa	IgM		IgG		IgE	
	Primary infection	Secondary infection	Primary infection	Secondary infection	Primary infection	Secondary infection
150	84.6	70.6	30.8	82.4	84.6	94.1
116	76.9	35.3	7.7	0	7.7	0
96	7.7	5.9	0	82.4	0	0
85	76.9	35.3	30.8	47.1	0	0
72	69.2	17.6	7.7	35.3	7.7	0
67			23.1	94.1	0	0
65	100	52.9	84.6	100	7.7	5.9
60	76.9	47.1				
55	76.9	64.7	69.2	94.1	23.1	33.3
48	7.7	0	0	0	0	0
45	30.8	11.8	15.4	64.7	7.7	0
38	100	94.1	30.8	82.4	7.7	33.3
28	61.5	47.1	53.8	82.4	38.5	23.5

This work paves the way for studying specific IgE responses with other HCMV recombinant proteins.

Acknowledgements

We thank Professor MP Landini for providing recombinant protein p52 (pUL44), Professor H. Bazin for the rat monoclonal antibodies LoHe10 and LoHe 17 and Professor JM Saint Remy for total serum IgE assays. Dr MA Vargas was supported by the AGCD and the Universidad Nacional de Colombia.

References

- Bazin, H. (1981) Production of rat monoclonal antibodies with the LOU rat not secreting IR983F myeloma cell line. *Prot. Biol. Fluids*. Peeters E (Ed.), 29th colloquium 1981, Pergamon Press, Oxford. 615–618.
- van der Bij, W., Torensma R, van Son W.J., Anema J., Schirm J. and Tegzess A.M. (1988) The rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leucocytes. *J. Med. Virol.* 25, 179–188.
- van den Berg, A.P., van der Bij, W., van Son, W.J., Anema J., Giessen M. van der Schirm, J. and Tegzess A.M. (1989) Cytomegalovirus antigenemia as a useful marker of symptomatic infection after renal transplantation — a report of 130 consecutive patients. *Transplantation* 48, 991–995.
- Fresard, A., Vindimian, M., Genin, C., Lepetit, J.C., Pozzetto, B. and Lucht, F. (1994) Serum IgE and human immunodeficiency virus (HIV) infection. *Pathol. Biol.* 42, 750–753.
- Geha, RS (1992) Regulation of IgE synthesis in humans. *J. Allerg. Clin. Immunol.* 90, 143–150.
- Israël-Biet, D., Labrousse, F., Tourani, J.M., Sors, H., Andrieu, J.M. and Even, P. (1992) Elevation of IgE in HIV-infected subjects: A marker of poor prognosis. *J. Allerg. Clin. Immunol.* 89, 68–75.
- Lagacé-Simard, J., Portnoy, J.D. and Wainberg, M.A. (1986) High levels of IgE in patients suffering from frequent secondary herpes simplex lesions. *J. Allergy. Clin. Immunol.* 77, 582–585.
- Landini, M.P., Re, M.C., Mirolo, G., Baldassarri, B. and La Placa, M. (1985) Human immune response to cytomegalovirus structural polypeptides studied by immunoblotting. *J. Med. Virol.* 17, 303–311.
- Lamy, M.E., Mulongo, N.K., Vargas, M., Pirson, Y. and Squiffet, J. (1994) Early diagnosis of CMV infection by detection of pp65 antigen in 91 renal transplant recipients. *Transpl. Int.* 7, 237–242.
- Landini, M.P. (1992) Antibody responses to human cytomegalovirus proteins. *Rev. Med. Virol.* 2, 63–72.
- Lazzarotto, T., Dalla Casa, B., Campisi, B. and Landini, M.P. (1992) Enzymelinked immunoabsorbent assay for the detection of cytomegalovirus IgM: comparison between eight commercial kits, immunofluorescence and immunoblotting. *J. Clin. Lab. Anal.* 6, 216–218.
- Ljungman, P. and Griffiths, P. (1993) Definitions of cytomegalovirus infection and disease. In: S. Michelson and S.A. Plotkin (Eds.), *Multidisciplinary Approach to Understanding Cytomegalovirus Disease*, Excerpta Medica, Amsterdam, pp. 233–237.
- Nielsen, S.L., Sorensen, I. and Andersen, HK. (1988) Kinetics of specific immunoglobulins M, E, A, and G in congenital, primary, recurrent cytomegalovirus infection studied by antibody-capture enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* 26, 654–661.
- Perelmutter, L., Potvin, L. and Phipps, P. (1979) Immunoglobulin E response during viral infection. *J. Allerg. Clin. Immunol.* 64, 127–130.
- Ripalti, A., Dal Monte, P., Boccuni, M.C., Campanini, F., Lazzarotto, T., Campisi, B., Ruan, Q. and Landini, M.P. (1994) Prokaryotic expression of a large fragment of the most antigenic cytomegalovirus DNA-binding protein (ppUL44) and its reactivity with human antibodies. *J. Virol. Meth.* 46, 39–50.
- Van Loon, A.M., van der Logt, J.T.M., Heesen, F.W.A., and van der Veen, J. (1985) Quantification of immunoglobulin E antibody to cytomegalovirus by antibody capture enzyme linked immunosorbent assay. *J. Clin. Microbiol.* 21, 558–561.
- Vercelli, D. and Geha, R.S. (1991) Regulation of IgE synthesis in humans: a tale of two signals. *J. Allergy. Clin. Immunol.* 88, 285–295.
- Weber, B., Braun, W., Tyralla, B., Hamann, A. and Doerr, H.W. (1992) Human cytomegalovirus (CMV) -specific immunoglobulin E as a serologic marker for CMV infection in immunocompromised patients. *Clin. Invest.* 70, 497–502.
- Weber, B., Stemler, A., Ernst, W., Scheurman, E.H., Braun, W. and Doerr, H.W. (1993) Improvement of aerological diagnosis of human cytomegalovirus infection in renal transplant recipients by testing for specific immunoglobulin E by ELISA. *Infection* 21, 158–163.
- Welliver, R.C., Wong, D.T., Sun, M., Middleton, E., Vaughan, R.S. and Ogra, P.L. (1981) The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. *N. Engl. J. Med.* 305, 841–846.
- Welliver, R.C., Wong, D.T., Middleton, E., Sun, M., McCarthy, N. and Ogra, P.L. (1982) Role of parainfluenza virus specific IgE in pathogenesis of croup and wheezing to infection. *J. Pediatr.* 101, 889–896.
- Wright, D.N., Nelson, R.P., Ledford, D.K., Fernandez-Caldas, E., Trudeau, W.L. and Lockey, R.F. (1990) Serum IgE and human immunodeficiency virus (HIV) infection. *J. Allergy Clin. Immunol.* 85, 445–452.
- Zipeto, D., Baldanti, F., Zella, D., Furione, M., Cavicchini, G., Milanese, G. and Gerna G (1993) Quantification of human cytomegalovirus DNA in peripheral blood polymorphonuclear leucocytes of immunocompromised patients by polymerase chain reaction. *J. Virol. Methods* 44, 45–56.