BANTAO Journal 2010; 8 (1): 44-49

BJ
BANTAO Journal

Original Article

HLA-A11 Increases the Risk for Post - Transplant Active Cytomegalovirus Infection in Kidney Graft Recipients

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Abstract

Introduction. Cytomegalovirus (CMV) infection remains one of the most common complications of kidney transplantation. The role of HLA system in CMV infection is still controversial. The aim of our study was to assess possible associations of particular HLA alleles with the occurrence of CMV infection in kidney transplant recipients.
Methods. A total of 101 kidney graft recipients positive for anti-CMV IgG prior to transplantation were included in the study. An individual was assigned as having CMV infection if elevated titers of IgM CMV antibodies were detected by ELISA. HLA-A, -B, -DR and -DQ typing was performed by serological and/or DNA-PCR based methods. Chi-square and Fischer's exact tests were used for data analysis. P< 0.01 was considered statistically significant.
Results. Active CMV infection occurred in 52 of the 101 transplanted patients. No significant differences in respect to gender, age, maintenance immunosuppressive regimen, anti-rejection and induction therapy were observed between patients with and without infection. A statistically significant association between the presence of HLA-A11 and development of active CMV infection was found (p=0.002). In addition, certain associations were close to the level of significance: the occurrence of infection was higher among patients possessing HLA–B16 (p=0.029) or HLA–DR13 (p=0.019) and lower among the carriers of HLA–DR11 (p=0.012).
Conclusions. Since the HLA system plays a key role in human immunity, it can be expected that some alleles may modulate the antiviral immune response. Our findings suggest that HLA-A11 may significantly increase the risk for CMV infection among kidney transplant recipients.

Keywords: cytomegalovirus (CMV) infection, HLA system, kidney transplantation

Introduction

Cytomegalovirus (CMV) infection is a serious complication after solid organ transplantation, significantly reducing patients and graft survival [1]. Numerous studies have tried to identify the risk factors for CMV infection and possible ways to avoid it. There are several well-recognized risk factors so far – immunosuppressive regimen, anti-rejection and induction therapy, CMV status of the recipient and the donor prior to transplantation. Patients negative for anti-CMV IgG before transplanttation receiving anti-CMV IgG positive allograft are at higher risk for development of CMV infection after transplantation [1]. However, we focused our study on patients positive for anti-CMV IgG before transplantation, as they represent the vast majority of candidates for organ transplantation, especially kidney transplantation [2]. Apart from these risk factors, many investigators stress the importance of the HLA system in the antiviral responses, especially against the CMV pathogen. Fan *et al.* reported that HLA-A2 and HLA-DR11 increase and HLA-B16 reduces the risk for CMV infection [3]. Kraat *et al.* associated HLA–DR7 with increased risk for CMV infection [4], whereas Gomez and coworkers rejected any DR7/CMV association [5]. Retierre *et al.* reported a statistically significant correlation between HLA-A11, HLA-A32 and HLA-DR11 and prevalence of the viral infection among solid organ recipients [6]. However, other investigators found HLA-A11 a protective factor in cytomegalovirus infection alongside with HLA-B51 [7]. To our best knowledge, there has been only one report linking CMV infection with DQ allele (HLA-DQ3) [8]. Obviously the reports concerning this topic are controversial and even conflicting. Nevertheless, some HLA alleles (HLA-A11, HLA-B16, HLA-DR11) can be traced in most of the studies. The aim of the present study was to assess possible associations of host genetic factors (particular HLA alleles) with CMV infection in kidney transplant recipients. No

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assessment was made in respect to the severity of the infection. Our results reveal highly significant association between HLA-A11 and CMV infection after transplanttation in patients positive for anti-CMV IgG prior to the operation.

Patients and methods

Patients

Out of 424 kidney transplant recipients in our transplant center, only those tested for HLA-A, -B, -DR and -DQ alleles and positive for anti-CMV IgG prior to transplantation were selected retrospectively and were included in the study (n=101). The patients were transplanted between 2000 and 2008 and were not selected in respect to the type of donor (living or cadaver donor).
Exclusion criteria: more than one kidney transplantation, CMV prophylaxis after the operation, CMV negative patients within one year after transplantation at the time of the study (December 2008), CMV-IgG negative recipients before the operation.
The patients were divided into two groups according to the presence of active CMV infection.

Maintenance immunosuppression

All patients were on triple immunosuppressive regimen – prednisolone, calcineurin inhibitor and cytostatic medication – azathioprine or mycophenolic acid derivatives. One hundred patients were treated with cyclosporine A and one patient received tacrolimus; all patients were treated with prednisolone. Out of seventy-two patients who received azathioprine, 10 were switched to mycophenolate acid derivatives. Mycophenolates initially after transplantation received 29 individuals. Cyclosporin A and tacrolimus doses were tailored according to their serum levels. Initial azathioprine doses were 3 mg/kg. Initial doses of prednisolone were 5-10 mg/kg in the first days and were tapered gradually to maintenance dose of 10 mg/daily at the third month after transplanttation. The doses of mycophenolic acid derivatives were standard (1000 mg b.i.d for mycophenolate mofetyl and 720 mg b.i.d. for mycophenolate sodium).

Induction and anti-rejection therapy

Of all 101 individuals, 16 patients were treated with anti-thymocyte globulin (Thymoglobulin) as induction or anti-rejection therapy. Thymoglobulin was administered for 7 to 10 days and doses were tailored based on the lymphocyte count. Twenty-two patients were treated with high doses of prednisolone for rejecttion, 9 patients received anti-CD25 preparation (basiliximab or daclizumab) as induction therapy.

Testing for CMV

Chemiluminiscence ELISA kit (Diasorin, LIAISON® hCMV serology line, USA) was used for determination of anti-CMV antibodies. A patient was regarded as CMV positive if elevated anti-CMV IgM levels were detected. Results in the gray zone were regarded as negative.

HLA typing

HLA-A and -B typing was performed by CDC method using magnetic beads for cell isolation and monoclonal typing trays (One Lambda, USA).When necessary DNA typing (PCR-SSP) was also performed. HLA-DR and -DQ alleles were determined by PCR-SSP technique (Olerup, Sweden).

Statistical analysis

Qualitative data were analyzed using Chi-square test and Fischer's exact test. The Mann–Whitney test was used for quantitative variable analysis (patients' age). SPSS 11.5 software was used for the statistics. In our study, values of $p < 0.01$ were considered statistically significant in the setting of relatively small number of cases.

Results

Gender and age

In the group with CMV infection (n=52), there were 14 females and 38 males, whereas the anti-CMV IgM negative group (n=49) consisted of 17 females and 32 males. No statistically significant association between gender and CMV prevalence was established (p=0.40). The difference in mean age between the two groups was also insignificant (39.62±11.96 for the CMV positive patients and 38.71±12.16 for the CMV negative ones, p=0.668).

CMV status before transplantation

As it has already been mentioned, all patients included in the study were positive for anti-CMV IgG antibodies prior to transplantation. In the CMV infection group, there were 25 IgG positive donors, one was IgG negative and for 26 donors no data were available. In the cases without infection in the post-transplant period, 21 donors were positive for CMV IgG antibodies and for 28 donors no data were available. Despite the missing data, no statistically significant relation was established in respect to the CMV status of the donors (p=0.369) and the development of active CMV infection in the post-transplant period.

Maintenance immunosuppression

Since all patients were treated with prednisolone and cyclosporine A (only one patient with tacroimus), these two medications were not assessed. The stress was laid on the use of mycophenolic acid derivatives and azathioprine. Among patients with infection in the post-

transplant period, 34 were receiving azathioprine, 19 mycophenolic acid derivatives, in 1 case azathioprine was substituted with mycophenolate preparation. Thirty-eight of the anti-CMV IgM seronegative patients were receiving

Table 1. Demographic and therapeutic characteristics*

Factor	CMV Infection–Positive (n=52)	CMV Infection–Negative (n=49)	P value
Patients' age	39.62±11.96	38.71±12.16	0.668
Patients' gender (male-to-female ratio)	38/14	32/17	0.400
Donor CMV status			
IgG seropositive	25	21	0.369
IgG seronegative	1	0	
n.a.	26	28	
Medications			
Azathioprine	34	38	0.179
Mycophenolic acid derivatives	19	20	0.661
Thymoglobulin	9	7	0.679
Pulse steroids	14	8	0.199
Anti-CD25	2	7	0.086

n.a. – not available; * Unless otherwise indicated, values are given as number of patients

aza thioprine, 20 mycophenolate, in 9 cases azathioprine was substituted for mycophenolate medication.

There was no significant association between CMV infection and azathioprine therapy (p=0.179) and between mycophenolic acid derivatives and CMV infection (p= 0.661) (Table 1).

Induction and rejection therapy

Of 52 patients with CMV infection, 9 were treated with thymoglobulin, 14 were on steroid pulse therapy due to rejection and in 2 cases anti-CD25 preparation was administered. In the CMV infection negative group, 7 patients received thymoglobulin treatment, 8 were on steroid pulse therapy and in 7 out of 49 recipients anti-CD25 induction was performed. Again, no statistically signifycant differences were detected for thymoglobulin, pulse steroid therapy, anti–CD25 medications (p=0.679, p= 0.199, p=0.086, respectively). The results are summarized in Table 1.

HLA/CMV infection associations

All HLA types detected in our patients were included in the analysis. We found that 14 (26.20%) out of 52 patient with active CMV infection after transplantation were HLA-A11 carriers (Figure 1). In the group without CMV infection post transplantation (n=49), only 2 (4.08 %) were HLA-A11 positive. Significant association was established (Fisher's exact test, p=0.002) between the occurrence of CMV infection and the presence of HLA-A11 in kidney recipients.

Many other HLA alleles were reported to influence the risk for CMV infection after transplantation – A2, A24, A32, B16, B51, DR7, DR11, DR13, DR15, DQ3. Therefore, we also analyzed the influence of these alleles in our cohort of patients. Except for HLA-A11, no other statistically significant association was established, according to the accepted level of significance (p<0.01). The data are summarized in Table 2.

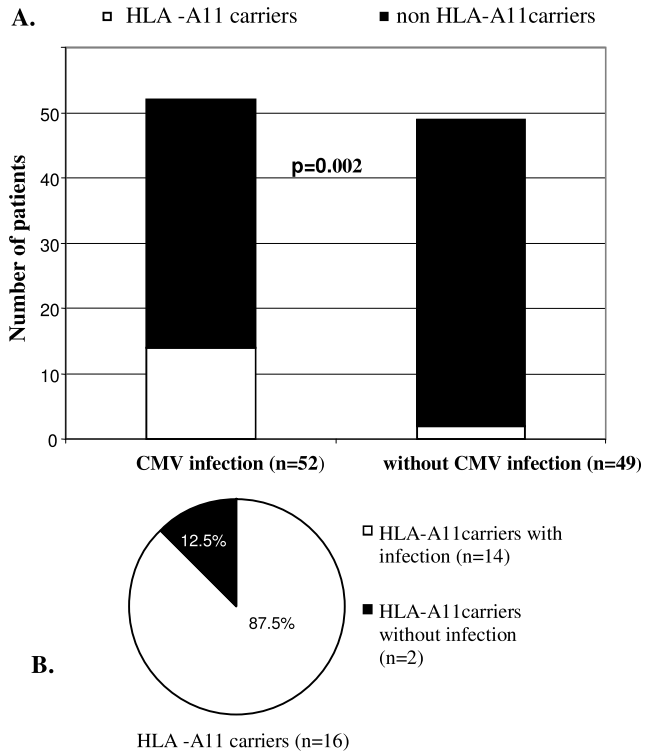

Fig. 1. HLA-A11 frequency in kidney graft recipients according to their CMV infection status after transplantation. A. In the patients group with active CMV infection 14 of 52 individuals carried HLA-A11, while only 2 of 49 recipients without infection were positive for this allele. B. Among the HLA-A11 positive renal transplant recipients (15.84% of all studied patients), active CMV infection was observed more frequently (87.5%) in comparison to the lack of infection (12.5%).

We have to admit that certain associations were close to

the level of significance ($p<0.01$): the occurrence of CMV infection was higher among patients possessing HLA–B16 (Fisher's exact test, p=0.029) or HLA–DR13 (Fisher's exact test, p=0.019) and lower among the carriers of HLA–DR11 (χ^2=6.249, p=0.012). All other investigated HLA alleles showed no associations with CMV infection prevalence after transplantation.

Table 2. HLA-A, -B, -DR, -DQ alleles and the occurrence of CMV infection after transplantation

HLA	CMV infection (n=52)	No infection (n=49)	Odds ratio	99% CI	P value*
A2	23	24	0.826	0.273 – 2.496	0.633
A11	14	2	8.658	2.866 – 26.158	0.002
A24	8	14	0.455	0.150 – 1.373	0.109
A32	2	3	0.613	0.203 – 1.853	0.672
B16	10	2	5.595	1.852 – 16.905	0.029
B51	12	10	1.17	0.388 – 3.535	0.745
DRB1*07	8	9	0.808	0.267 – 2.441	0.69
DRB1*11	13	24	0.347	0.115 – 1.049	0.012
DRB1*13	13	4	4.145	1.372 – 12.522	0.019
DRB1*15	7	7	0.933	0.309 – 2.820	0. 905
DQB1*03	32	27	1.304	0.432 – 3.939	0.512

*P values estimated by Chi-square test and Fisher's exact test

In order to exclude the influence of the recognized risk factors for CMV infection we analyzed the HLA–A11 positive patients only. No statistically significant differences were established in respect to gender, age, immunosuppressive therapy between HLA–A11 positive patients with CMV infection and HLA-A11 positive recipeents without infection. The results are shown in Table 3.

Table 3. HLA–A11 positive patients - demographic and therapeutic characteristics*

Factor	CMV Infection–Positive (n=14)	CMV Infection–Negative (n=2)	P value
Age	36.14±12.39	36.5±4.95	0.691
Gender (male-to-female ratio)	9/5	1/1	0.696
Donor CMV status			
IgG seropositive	5	1	1.00
IgG seronegative	1	0	
n.a.	8	1	
Medications			
Azathioprine	6	2	0.467
Mycophenolic acid derivatives	8	0	0.467
Thymoglobulin	1	0	1.00
Pulse steroids	5	0	1.00
Anti-CD25	1	0	1.00

n.a. – not available; * Unless otherwise indicated, values are given as number of patients

Discussion

Human cytomegalovirus is a commonly occurring herpes virus present in 50–90% of the population, largely without pathological consequences. However, in clinically immunodeficient or immunosuppressed patients, unhindered virus-associated replication can occur and CMV is one of the most important pathogens affecting renal transplant recipients [9]. Obviously the more risk factors for CMV infection we are acquainted with, the less hazardous our immunosuppression will be. Therefore, the major goal of investigators is to identify the risk factors for CMV infection and establish the correct prophylaxis. It is well known that certain HLA alleles are associated with autoimmune and infectious diseases [10-14]. There is accumulating data showing the impact of this genetic system for the anti-CMV responses in organ transplanted patients, especially those genes encoding the MHC class I molecule, which play a key role in the antiviral defense [3,6,7].

Three CMV peptides were found to be presented by MHC class I molecules–glycoprotein B, immediate early protein 1 and pp65 [6]. The complex MHC class I – viral peptide activates the CD8+ cytotoxic T-lymphocytes (CTL), thus initiating the antiviral response. Our results demonstrated significant association (p=0.002) between HLA-A11 and CMV infection after kidney transplanttation among anti-CMV IgG positive recipients prior to transplantation. HLA-A11 frequency among the patients from the National KidneyWaiting List is consistent with that in the Bulgarian population (0.0758 vs. 0.0686) [15]. However, higher percentages of patients (15.84%) in our study group were carriers of this allele and most of them developed active CMV infection in the post-transplant period. The established increased risk of active CMV infection in HLA-A11 positive individuals re-

ceiving renal allograft is in agreement with the findings of Retiere *et al*. [6] for kidney transplant recipients from the French population. However, the data of Chinese authors [3] suggested that HLA-A11, which is a common allele in China (varying between 12.1% and 38% across the different Chinese ethnic groups; *www. Allelefrequencies.net),* might escape serious CMV infection in liver transplanted patients. It should be mentioned that in this study we did not assess the HLA associations with the severity of CMV infection. Recent investigations have demonstrated that a differing efficiency of the antiviral CTL responses may be restricted by different HLA allele subtypes both in healthy CMV positive volunteers [16, 17] and hematopoetic stem cell recipients [17]. Taking into consideration these findings we can speculate that the discrepant results for CMV susceptibility of HLA-A11 carriers observed in different ethnic groups and geographical location may be due to different population specific HLA-A11 allele subtypes. However, this explanation is not supported by the data for the Bulgarian, French and Chinese populations in which the HLA-A*11011 is the most frequent HLA-A11 allele *(www.allelefrequencies.net*). Thus, it is reasonable to suggest that the discrepancies observed by different authors might be caused by different human CMV genotypes in distinct geographic regions. In this connection it should be noted that HLA-A11-positive Caucasians frequently respond to two immunodominant epitopes of the nuclear antigen EBNA3B [18]. While mutations within these EBV epitopes, observed in two different highly HLA-A11-positive populations (Papua New Guinea and Southern Chinese), clearly affected antigenicity and did not mount detectable A11-restricted CTL responses [18, 19]. Thus, the viral subtype prevalence in the different geographic regions should be also taken into consideration.

Our findings have also revealed close to the adopted level of significance ($p<0.01$) associations between DRB1*11 ($p=0.012$) and DRB1*13 ($p=0.019$) and active infection after transplantation, with data indicating that DRB1*11 may be a protective allele. The MHC class II restricted CD4+ T-cell immune responses play an important role in antiviral defense by enhancing antibody production and maintaining the number of cytotoxic T cells [20]. Japanese investigators reported association between decreased production of anti-CMV antibodies in patients with certain HLA types (HLA-DR15) and increased in others (HLA-DR9) [21]. In addition, Ishibashi *et al*. [22] found that fewer subjects with HLA-DR10 and DR11 had antibodies against CMV gH than did those without HLA-DR10 and DR11, indicating that these alleles may be associated with fewer/non-responders for strain-specific neutralizing antibodies. Contrary to the findings of other authors [3,6], our data suggest that HLA-DR11 may be protective for the development of active CMV infection in the Bulgarian kidney transplant recipients, since the statistical significance ($p<0.012$) of this association was very close to the accepted level of significance ($p<0.01$). In addition, significant independent associations between some CMV genotypes and particular HLA alleles in renal transplant recipients were observed by Retiere *et al*. [6], with higher gB1 and lower IE1-2 genotype frequencies in DR11 carriers. Again, population specific HLA-DR allele subtypes and/or CMV strains could be suggested as an explanation for the reported differences. Further investigations in larger groups of patients will be necessary in order to clarify the role of HLA class II antigens in CMV infection in kidney transplanted patients from our population. Moreover, the combined effect of HLA class I and class II alleles should also be elucidated.

Our study has an important link with the routine clinical practice, as we have focused on patients positive for anti-CMV IgG antibodies before transplantation that represent the vast majority of kidney transplant candidates. In these recipients, CMV infection after transplantation is due either to reactivation of a past infection or newly acquired infection with a different CMV genotype.

In everyday clinical practice our findings can be implemented in two major directions – reducing the immunosuppressive regimen in HLA-A11 carriers or CMV prophylaxis. As the reduction of the immunosuppressive therapy increases the risk for rejection, the second option – CMV prophylaxis seems to be the more realistic approach.

We must admit, however, that it is a long way till certain HLA alleles can be recognized as risk factors for CMV infection. The main reason for this are the controversial results, which can be partly explained with differrent cohorts of patients investigated, population specific HLA genetic background of each geographic region leading to different allele frequencies. Other factors associated with controversial results such as co-influence of several HLA alleles, infection with different subtypes of human cytomegalovirus or social environmental and economic ones in the different countries cannot be excluded, too. Perhaps larger studies in different ethnic groups are needed to elucidate the association between certain HLA alleles and CMV infection, which in turn might design individual treatment strategies in transplant organ recipients.

Acknowledgement. We would like to thank Mrs Kremena Mihaylova – Filipova for providing us the statistical calculations.

Conflict of interest statement. None declared.

References

1. Patel R, Paya CV. Infections in solid-organ transplant recipients. *Clin Microbiol Rev* 1997; 10: 86–124.
2. McKay DB, Milford EL, Tolkoff-Rubin NE. Clinical Aspects of Renal Transplantation. In: Levine SA, editor. *Brenner and Rector`s The Kidney, 6th edition*. Philadelphia: W.B. Saunders Company; 2000. pp. 2542 – 2590.
3. Fan J, Meng XQ, Yang MF, *et al*. Association of cytomegalovirus infection with human leukocyte antigen genotypes in recipients after allogeneic liver transplantation. *Hepatobiliary Pancreat Dis Int* 2006; 5: 34-38.
4. Kraat YJ, Christiaans MH, Nieman FH, *et al*. Risk Fac-

tors for cytomegalovirus infection and disease in renal transplant recipients:HLA-DR7 and triple therapy. *Transpl Int* 1994; 7: 362–367.
5. Gomez E, Aguado S, Melon S, *et al*. Absence of association between HLA-DR7 and cytomegalovirus infection in renal transplant patients. *Lancet* 1993; 341: 1480–1481.
6. Retiere C, Lesimple B, Lepelletier D, *et al.* Association of glycoprotein B and immediate early-1 genotypes with human leukocyte antigen alleles in renal transplant recipients with cytomegalovirus infection. *Transplantation* 2003; 75: 161–164.
7. Chen Y, Rocha V, Bittencourt H, *et al*. Relationship between HLA alleles and cytomegalovirus infection after allogenic hematopoieticstem cell transplant. *Blood* 2001; 96: 500–501.
8. Varga M, Rajczy K, Telkes G, *et al*. HLA-DQ3 is a probable risk factor for CMV infection in high-risk kidney transplant patients. *Nephrol Dial Transplant* 2008; 23: 2673 – 2678.
9. Fishman JA, Emery V, Freeman R *et al*. Cytomegalovirus in transplantation — challenging the status quo. *Clin Transplant* 2007; 21:149–158.
10. Ghodke Y, Joshi K, Chopra A, Patwardhan B. HLA and disease. *Eur J Epidemiol* 2005; 20: 475-488.
11. Lie BA, Thorsby E. Several genes in the extended humanMHC contribute to predisposition to autoimmune diseases. *Curr Opin Immunol* 2005;17: 526–531.
12. Martin MP, Carrington M. Immunogenetics of viral infections. *Curr Opin Immunol* 2005; 17: 510–516.
13. Vejbaesya S, Songsivilai S, Tanwandee T, *et al.* HLA association with hepatitis C virus infection. *Human Immunol* 2000; 61: 348-353.
14. Singh R, Kaul R, Kaul A, Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. *World J Gastroenterol* 2007; 13: 1770-1787.
15. Naumova E, Ivanova M. Major Histiocompatibility Complex – facts, hypothesis, application in medicine, 1st edition, Sofia: Publishing house "Litze"; 2006. pp. 35-36.
16. Burrows JM, Wynn KK, Tynan FE, *et al*. The impact of HLA-B micropolymorphism outside primary peptide anchor pockets on the CTL response to CMV. *Eur J Immunol.* 2007; 37: 946-953.
17. Giest S, Grace S, Senegaglia AC, *et al*. Cytomegalovirus-specific CD8(+) T cells targeting different HLA/peptide combinations correlate with protection but at different threshold frequencies. *Br J Haematol* 2010; 148 (2):311-322..
18. De Campos-Lima PO, Gavioli R, Zhang QJ, *et al*. HLA-A11 epitope loss isolates of Epstein-Barr virus from a highly A11+ population. *Science* 1993; 260: 98-100.
19. Midgley RS, Bell AI, Yao QY, *et al*. HLA-A11-restricted epitope polymorphism among Epstein-Barr virus strains in the highly HLA-A11-positive Chinese population: Incidence and immunogenicity of variant epitope sequences. *J Virol* 2003; 77: 11507-11516.
20. Bitmansour A, Waldrop SL, Pitcher CJ, *et al*. Clonotypic structure of the human CD4+ memory T cell response to Cytomegalovirus. *J Immunol* 2001; 167: 1151-1163.
21. Wada K, Mizuno S, Ohta H, *et al*. Immune response to neutralizing epitope on human cytomegalovirus glycoprotein B in Japanese: correlation of serologic response with HLA-type. *Microbiol Immunol* 1997; 41: 841–845.
22. Ishibashi K, Tokumoto T, Shirakawa H, *et al*. Association between antibody response against cytomegalovirus strain-specific glycoprotein H epitopes and HLA-DR. *Microbiol Immunol* 2009; 53: 412-416.