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Standard Review

The HIV-based host derived R7V epitope; functionality of antibodies directed at it and the predicted implications for prognosis, therapy or vaccine development

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Host protein beta-2 microglobulin (β2m) is incorporated into the HIV-1 coat during budding. Individuals not progressing to AIDS produce antibodies directed to an epitope contained in β2m which is designated R7V. These antibodies increased with duration of HIV-infection in non-progressor patients and protected against HIV replication. Purified R7V antibodies neutralized different HIV-1 isolates and did not bind to human cells. In individuals progressing to AIDS or using antiretroviral treatment, a lower prevalence of R7V antibodies was observed. This review summarizes findings on the R7V epitope and antibodies directed at it. Suggestions are also made as to necessary research on R7V which may clarify the importance of this epitope in HIV therapy, prognosis or vaccine development.

Key words: R7V, epitope, antibodies, β2m, HIV, ELISA.

Table of content

INTRODUCTION

HIV, the retrovirus known for disabling CD4 expressing T cells and causing immune system dysfunction, incorporates

host cell proteins into its envelope during budding. These virus incorporated host proteins can be bystanders, assist in the life cycle and viral ability to avoid immune system detection, while some of these proteins retain their functional ability or engage in responses that are detrimental to the pathogen. The host immune response to these cellular proteins when they are presented as virus-associated particles allows room for investigating them as prognostic markers, therapeutic tools or potential vaccine candidates. Existing AIDS prognostic markers like CD4 cell count and viral load are not infallible and better therapies against HIV is still under development because of shortcomings in existing treatment regimes. In addition, the uneven record of success of HIV vaccine strategies leaves room for considering host proteins incorporated by the virus as a radically different means of generating protective immunity.

Beta-2 microglobulin (β2m) is one of the host proteins incorporated into the envelope of HIV-1 and under discussion in this review are the possible uses of antibodies to R7V, an epitope within this protein. R7V is incorporated into the envelope of HIV-1 and located on the exterior surface (Le Contel et al., 1996). This epitope has been suggested as possible vaccine target (Le Contel et al., 1996; Galéa et al., 1999 a and b; Chermann, 2001; Haslin and Chermann, 2007b) and antibodies induced by it as prognostic (Galéa et al., 1996; Chermann, 2001; Ravanini et al., 2007; Kouassi et al., 2007; Sanchez et al., 2008) or therapeutic markers (Haslin and Chermann, 2002, 2004 and 2007b; Haslin et al., 2007a). Data that lead to these suggestions are reviewed here. Because reports on R7V are limited (only 11 articles in peer reviewed journals and a number of patents), data contained in posters (4 posters reviewed by a scientific committee and presented at international conferences) as well as personal communications with researchers (Webber, 2009) are also referenced to provide a complete picture of work done on this epitope thus far.

Cellular proteins in HIV

Cellular or host proteins can be incorporated by HIV-1 either on its surface or inside the viral lipid envelope (Ott, 2008; Figure in Galéa et al., 1999b). Some host proteins present in the virus retain their functional ability and can affect infectivity, tropism and pathogenesis. According to Ott (2008) there are three possible ways in which HIV-1 incorporates cellular proteins inside or on its surface. Most of the cellular proteins incorporated by the virus are taken up as simple bystanders because of their close proximity during the budding process. The presence of these non-specifically incorporated proteins provides important information about the local environment where HIV-1 assembles and the site of budding. The second possible mechanism of incorporation involves host proteins that act as partners in the assembly and budding process and these are incorporated by interacting with one of the viral proteins. Lastly, HIV-1 can hijack cellular proteins by incorporating them for a post-assembly step where they act as captives helping the virus replicate or evade the immune system (Ott, 1997 and 2008). Selected cellular proteins detected in HIV-1 as well as their role in favour of the virus are detailed by Ott, 1997 and 2008. Since β2m is part of the major histocompatibility complex (MHC) class I, the assumption is that this protein is incorporated into HIV's envelope in the same manner as MHC class I. From information provided in the review by Ott, 2008 it seems that HIV incorporates this protein as a bystander as there is no evidence that a specific HIV protein acts as a binding partner to bring the protein complex into the viral particle.

Beta-2 microglobulin

β2m is a 12 kDa, 99 amino acid protein which is non-covalently bound to the 45 kDa heavy chain of the MHC class I molecule (Roitt et al., 2001) where it is essential for expression of this molecule. MHC class I plays a central role in the immune system, is omnipresently expressed and binds peptide antigens for presentation to the $CD8^+$ T-cells (Rosano et al., 2005) during the initiation of a cellular immune response. β2m is also associated with CD 1 (a protein related to MHC class I) for presentation of lipids, glycolipids and lipid antigens to T-cells (Roitt et al., 2001). Because β2m is expressed in almost all nucleated cells (Arthur et al., 1992), it can be found in all potential virus target cells. This protein along with neopterin and other serum and cellular markers that correlate with clinical progression of HIV disease (Fahey et al., 1990; Hofmann et al., 1990; Melmed et al., 1989) when found free in a variety of physiological fluids (Rosano et al., 2005), also serve as indicators of the degree of immune activation.

Beta-2 microglobulin as vaccine target

Arthur et al. (1992); Ott (1997) and Haslin et al. (2002) confirmed β2m as one of the cellular proteins incorporated

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Abbreviations: AIDS, acquired immunodeficiency syndrome; **ARV,** antiretroviral; **β2m,** beta-2 microglobulin; **CMV,** cyto-megalovirus; **CTL,** cytotoxic T-lymphocyte; **ELISA,** enzyme-linked immunosorbent assay; **HAART,** highly active anti-retroviral therapy; **HIV,** human immunodeficiency virus; **HLA,** human leukocyte antigen; **LTNP,** long term non-progressor; **MAbs,** monoclonal antibodies; **MHC,** major histocompatibility complex; **OD,** optical density.

into the surface of HIV-1. Monoclonal antibodies (MAbs) directed to β2m were able to immunoprecipitate intact viral particles and inhibit the life-cycle of HIV (Arthur et al., 1992). These data suggests β2m to be an integral and functioning part of the HIV-1 surface, involved in the process of HIV-infection and pathogenesis (Hoxie et al., 1987; Devaux et al., 1990; Corbeau et al., 1990; Arthur et al., 1992; Le Contel et al., 1996). β2m is immunogenic only when exposed on the viral surface and not when it is part of the human leukocyte antigen (HLA)/MHC class I at the host cell surface or when it is part of circulating β2m. MAbs against β2m reacts with free urinary β2m (Liabeuf et al., 1981) but not with the protein when it is associated with cell surface proteins.

To prevent binding between HIV-1 and a host cell, it may be worth considering targeting β2m in vaccine design and possibly avoiding the problems associated with the variability of viral proteins (Galéa et al., 1999b; Haslin et al., 2002). However it is only possible to consider such a vaccine approach if epitopes in the cellular determinant used will only be exposed when it is carried away by the extracellular infectious agent, or the epitopes are nonimmunogenic in their natural presenta-tion by the cell and is modified when it is presented at the surface of the virion (Chermann et al., 2000). This appears to be the case with epitopes in β2m. Studies demonstrating an inability of the potential vaccine antigen to induce autoimmune responses need to be designed. β2m based vaccine formulas appear to be under investigation as is evident in the references used in the patent by Chermann et al. (2000).

Most HIV protein-vaccine strategies to date focused on the variable viral envelope proteins, especially the V3 loop of gp120. Hewer and Meyer (2004 and 2005) hypothesized that exploiting the advantageous properties of V3 loop peptides and at the same time acounting for the variability of this region would aid in developing an effective vaccine component for inducing broadly cross reactive neutralizing antibodies. Addressing variability by novel synthesis induced viral strain specific immune responses with some amount of cross-reactivity which underscores the need for circumventing viral variability by other means.

The R7V epitope of beta-2 microglobulin

A seven amino acid epitope in β2m was shown to be present at the surface of divergent HIV isolates by Le Contel et al. in 1996. These authors studied several short overlapping peptides derived from β2m for their ability to reverse the neutralizing action of MAbs directed to the protein. Among the tested peptides, the heptamers R7V (Arg-Thr-Pro-Lys-Ile-Gln-Val), S7K (Ser-Gln-Pro-Lys-Ile-Val-Lys) and F7E (Phe-His-Pro-Ser-Asp-Ile-Glu) were efficient in reversing the action of these MAbs, with R7V being the most efficient. The R7V peptide consists of fewer hydrophobic (PIV) than hydrophilic (RTKQ) amino acids. The tertiary structure of isolated human β2m is described as an antiparallel β-barrel fold (Rosano et al., 2005). Multiple Protein Sequence Analysis programs, including DeepView/Swiss-Pdb Viewer 3.7 (SP5), describe percentages of the 7 amino acids in the R7V peptide as either a random coil or an extended strand which are regions that could form part of a β-barrel (PDB entry 1LDS; Trinh et al., 2002).

Polyclonal antibodies detected using an R7V peptide as antigen

Antibodies directed to R7V were detected in HIV-infected individuals, primarily asymptomatic and long term infected patients naïve of treatment. In studies done by Galéa et al. (1996) and Sanchez et al. (2008), R7V antibodies were present in the majority of asymptomatic patients (HIV seropositive for 5 - 12 years, stage A1-A2 according to the 1993 CDC classification, Galéa et al., 1996; class A according to the 1993 CDC classification and naïve of highly active antiretroviral therapy, HAART, Sanchez et al., 2008) and long-term infected patients (infected for more than 10 years without HAART, Sanchez et al., 2008). The highest prevalence of R7V antibodies were found in non-progressor patients compared to progressors who go on to develop AIDS with associated CD4 cell decreases and viral load increases (Galéa et al., 1996; Sanchez et al., 2008, Table 1). R7V antibodies were also found in individuals who were not infected with HIV but at lower levels (Galéa et al., 1996; Sanchez et al., 2008, Table 1). This is interesting because R7V is believed to only be immunogenic when incorporated into the HIV envelope and the virus was absent in these patients. These antibodies could be cross-reactive or perhaps the HIV negative individuals were infected by other enveloped viruses containing host derived R7V-like epitopes (more detail in paragraphs to follow).

The study done by Galéa and colleagues (1996) used an “in-house” enzyme-linked immunosorbent assay (ELISA) and the authors calculated concentrations of R7V antibodies from a standard curve while Sanchez et al. (2008) used the anti-R7V ELISA from Ivagen (Bernis, France) and determined the antibody ratio by normalizing the optical density (OD) value for the sample with the OD value for the internal calibrator. The Ivagen ELISA developed for detection of R7V in human serum and/or plasma of individuals confirmed as being seropositive for HIV-infection is not commercially available.

The Sanchez et al. (2008) study contained several groups; A (201 HIV negative, 160 HIV positive on treatment and 88 HIV positive untreated patients from USA), B (177 asymptomatic and 131 symptomatic HIV positive patients from USA) and C (45 untreated Italian non-progressor patients infected with HIV-1). From the

Table 1. Summary of studies reporting on the presence of R7V antibodies in HIV-infected and uninfected individuals. HIV-1 subtypes listed were inferred from locations where samples were collected since this information was not always stated.

Percent R7V antibody producing individuals				Total	HIV-1 subtype/country	References
HIV Positive vs. HIV negative						
%	n	%	n			
53.7	95	32.0	69	164	-	Galéa et al., 1996[3,4]
53.2	248	5.5	201	449	(B) USA	Sanchez et al., 2008[4,7]
33.5	507	3.0 [2]	201	708	(B) USA	Haslin and Chermann, 2007b[1,3,4]
9.1[7]	33	0.0	10	43	(B) Turkey	Ergünay et al., 2008[4,7]
Asymptomatic vs. Symptomatic						
%	n	%	n			
59.0	63	38.0	8	71	(B) Italy	Ravanini et al., 2007[1,4]
59.1	22	13.1	61	83	(A) Cameroon	Tagny et al., 2007[1,4]
42.0	36	9.0	53	89	(A) Ivory Coast	Kouassi et al., 2007[1,4]
64.4[5]	177[5]	35.1	131	308	(B) USA	Sanchez et al., 2008[4]
35.7[6]	29[6]	31.8[6]	22[6]	51	-	Galéa et al., 1996[2,3,4]
Treatment naïve vs. Treatment						
%	n	%	n			
67.0	45	35.0	17	62	(B) Italy	Ravanini et al., 2007[1,4]
56.8	88	21.3	159	247	(B) USA	Haslin and Chermann, 2007b[1,3,4]
38.0	50	2.0	50	100	(A) Ivory Coast	Kouassi et al., 2007[1,4]
71.6	88	43.1	160	248	(B) USA	Sanchez et al., 2008[4]
CD4 cell count:						
> 200 cells/µl vs. 0-200 cells/µl						
%	n	%	n			
59.1	22	13.1	61	83	(A) Cameroon	Tagny et al., 2007[1,4]
Years of HIV-infection (treatment naïve):						
		%	n			
< 5 years		14.3	-	-	(B) USA	Haslin and Chermann, 2007b[1,3,4]
5 - 10 years		50.0	-	-		
> 10 years		68.3	-	-		
0 - 5 years		~ 40.0	-	-	(B) USA	Sanchez et al., 2008[4]
5 - 10 years		~ 69.0	-	-		
10 - 20 years		~ 80.0	-	-		
< 5 years		68.0	41	62[8]	(B) Italy	Ravanini et al., 2007[1,4]
5 - 10 years		31.0	13			
≥ 10 years		63.0	8			

third study group Sanchez et al. (2008) noticed a direct correlation between R7V antibody ratio and viral load. On the other hand, no correlation between the R7V antibody ratios and the CD4 T-cell count was detected. Sanchez et al. (2008) also observed a higher prevalence of R7V anti-bodies in untreated patients (71, 6% n = 88) compared to patients on HAART (43.1% n = 160), Table 1. When treatment was considered successful, these authors re-

Table 1. Continues.

Ethnic groups:	%	n			
African Americans	49.5	106	501[2]	(B) USA	Haslin and Chermann, 2007b[1,3,4]
Haitians	38.0	100			
Asians	31.6	95			
Caucasians	26.2	84			
Indians	25.4	67			
Hispanics	22.4	49			
Sex:	%	n			
Males	55.0	61	106	(B) USA, African American	Haslin and Chermann, 2007b[1,3,4]
Females	44.4	45			

[1]Conference proceedings. 3rd South African AIDS Congress, Durban, June 2007.
[2]Calculation errors: The authors stated that 10 of 201 subjects equal 3%. However, 10 of 201 subjects equal 5%. Adding all the ethnic groups equals 501, 507 is stated as the total number by the authors.
[3]Galéa et al. (1996) and Haslin and Chermann (2007b) performed neutralizing assays with the R7V antibodies.
[4]The majority of studies were performed using the anti-R7V ELISA from Ivagen (Bernis, France) with the exception of Galéa et al. (1996) who used an "in-house" ELISA.
[5]Additional results in Sanchez et al. (2008): Long-term infected (more than 10 years) and naïve of treatment. Presence of R7V antibodies: ~ 80%, n not mentioned.
[6] Error: n = 29 stated in the text and n = 28 stated in the summary. Additional results in Galéa et al. (1996): presence of R7V antibodies in progressors: ~ 9% (n = 44).
[7]In Ergünay et al. (2008) all positive patients were on treatment. In Sanchez et al. (2008) 160 patients were on treatment and 88 patients were naïve of treatment.
[8]A sample number of 63 are stated in the poster. 18 of 63 patients were not on treatment.

ported a total disappearance in R7V antibodies for 77% (n = 21) of the patients. During antiviral treatment there is a decrease in newly formed virus particles budding from the host cell, it is therefore possible that fewer viruses containing the R7V epitope are produced in treated individuals. Sanchez et al. (2008) hypothesized that the R7V epitope may no longer be exposed on the virus and thus not visible to the host immune system after successful treatment and therefore suggested that the anti-R7V ELISA from Ivagen was better adapted to the detection of R7V antibodies in asymptomatic patients, still naïve of treatment.

Ergünay and colleagues (2008) following a smaller study (33 HIV positive and 10 HIV negative compared to the 160 patients on HAART in the Sanchez et al., 2008 study) also reported the presence of R7V antibodies in HIV-infected individuals on HAART (Table 1). Only 9.1% of 33 HIV positive individuals on treatment exhibited R7V antibodies and there was no correlation between the presence of the antibodies and disease progression. The Ergünay et al. (2008) report is in Turkish and the English abstract did not provide a description of the patients' disease status or duration of infection. It is difficult to directly compare the study done by Ergünay et al. (2008) and Sanchez et al. (2008) because the latter study used an HIV positive test group including patients both on HAART and not on treatment whereas the HIV positive test group in Ergünay`s study (2008) were all on HAART. The overall agreement between the works done by these groups is a decrease in prevalence of R7V antibodies in the presence of treatment.

A study reporting on the prevalence of the R7V antibodies in HIV-1 infected individuals on HAART was done by Professor Lynne Webber from the Department of Medical Virology, University of Pretoria, South Africa and presented at the HIV and AIDS Research symposium at the University of Pretoria in February 2009 (Webber, 2009). The prognostic applicability of the anti-R7V ELISA from Ivagen (Bernis, France) was examined using a cohort of 25 HIV-infected patients on HAART. Nine participants were classified as long term non-progressors (LTNPs), defined as patients free of HIV-1 related disease and displaying stable CD4+ T-lymphocyte counts (> 200 cells/µl for more than 10 years). The results indicated that 40% of all the HIV-infected patients and 56% of the LTNPs tested positive for R7V antibodies. Twenty eight percent of all the HIV-infected patients and 44% of the LTNPs were considered doubtful (data collected could not be classified as either positive or negative for R7V antibodies).

R7V Antibody and cross-reactivity

Data collected by Sanchez et al. (2008) demonstrated cross-reactivity of antibodies from individuals infected with other enveloped viruses when using the anti-R7V percent positive results in this study were limited. It is

possible that individuals not infected with HIV but infected with other enveloped viruses which may also incorporate β2m in their membrane and also expose the R7V epitope, could produce antibodies to this epitope. Sanchez et al. (2008) observed that a few individuals uninfected with HIV or infected with the enveloped viruses causing mono-nucleosis or rubella gave positive R7V antibody results using the Ivagen ELISA. Three of thirteen individuals (23.0%) infected with mononucleosis and six of eleven (54.5%) individuals infected with rubella responded positive for R7V antibodies (antibodies in the sera were able to bind the R7V antigen in an ELISA). Higher sample numbers are obviously needed to validate these data. Also these antibodies were not tested for an ability to neutralize or precipitate HIV-1 (which are properties of actual R7V antibodies) so it is possible that this response was due to some interference or cross-reactive antibo-dies. There is evidence that cytomegalovirus (CMV, McKeating et al., 1987) and HTLV (Hoxie et al., 1987) incorporate β2m. Since R7V is part of β2m this could mean that individuals infected with viruses other than HIV could test positive for R7V antibodies. However the acquisition of β2m by HIV and CMV differs. CMV acquires β2m after budding from the cell (in a non-HLA-like manner) but still binds MAbs to β2m (Grundy et al., 1987; Tysoe-Calnon et al., 1991; Le Contel et al., 1996). There is not enough evidence to form an opinion on whether CMV (containing β2m) could not induce an R7V like antibody response during infection.

Data collected using assays other than ELISA

Most studies referred to here present conclusions based on ELISA data only (where an R7V peptide was used as antigen). Whether the polyclonal antibodies believed to be R7V antibodies could neutralize or precipitate HIV-1 is not always indicated. Neutralization or precipitation of HIV-1 serves as a means of verifying an actual R7V antibody response. Better validation would be to purify the antibodies before use in either assay. Neither Galéa et al. (1996), Sanchez et al. (2008) nor Ergünay et al. (2008) performed precipitation assays. Of these three studies only Galéa et al. (1996) demonstrated that the presence of R7V antibodies correlated with neutralization of various divergent HIV strains. A study done by Xu et al. (2002) used both ELISA and precipitation to investigate the prevalence of R7V antibodies in HIV-infected patients. However, that data is not referred to in this review because the article is in Chinese and the English abstract does not give information about sample numbers or percentages of R7V antibodies. Since R7V antibodies neutralized divergent HIV-1 strains and this neutralization was reversed by addition of R7V peptide (Le Contel et al., 1996). Galéa et al. (1996) hypothesized that R7V antibodies found in patients could have equivalent neutralizing activity as that observed *in vitro*. Extensive experiments with the two main target cells of HIV-1 infection, peripheral blood lymphocytes and blood derived macrophages, have shown that different T-lymphotropic as well as macrophage-tropic HIV-1 strains was neutralized by β2m MAbs (Le Contel et al., 1996).

Evidence for R7V as potential vaccine target or therapeutic tool

Purified R7V antibodies from sera of rabbits injected with the peptide as well as in sera of non-progressor patients (HIV-1 subtype of infection virus not mentioned) precipitated and neutralized HIV-1 subtypes A, B, C, D and F (Galéa et al., 1999a). The results obtained by Galéa et al. (1999a) is supported by the studies done by Xu et al. (2002) who stated that R7V antibodies were found to inhibit the replication of HIV. Xu et al. (2002) also suggested that R7V antibodies prevent the virus from entering target cells by interfering with the binding of HIV to the co-receptors (CCR5 or CXCR4) of the target cell. In addition, human R7V IgG neutralized virus strains resistant to antiviral drugs and inhibited infection of cells by laboratory as well as primary viral isolates (Galéa et al., 1999b). Furthermore, studies done by Haslin and Chermann (2007b, poster presentation) showed R7V antibodies from HIV-1 subtype B infected individuals to be able to neutralize HIV-1 subtype D. Collectively the presence of R7V antibodies in serum from HIV-infected individuals from different geographic areas suggests that R7V is naturally immunogenic and escapes variability and flexibility observed with the viral proteins in the HIV envelope (Galéa et al., 1999a; Chermann, 2001). These studies suggest R7V-like epitopes to have a potential role in an HIV/AIDS vaccine and the R7V antibodies in the treatment of patients in failure of HAART.

Production of R7V antibodies with antiviral properties has been demonstrated by Haslin and Chermann (2004) and Haslin et al. (2007a). The antibodies were produced by infection of insect cells with a recombinant baculovirus in which the gene corresponding to the R7V antibody was introduced after isolation from EBV-immortalized B lymphocytes of non-progressor patients. These antibodies have been shown to be able to neutralize various clades of HIV-1 including drug-resistant viruses and should therefore be taken into consideration as therapeutic tools (Haslin and Chermann, 2004; Haslin et al., 2007a). There are several studies reporting on the prevalence of the R7V antibodies in HIV-1 infected individuals which were presented at the 3rd South African AIDS Congress in Durban in June 2007 (Ravanini et al., 2007; Haslin and Chermann, 2007b; Tagny et al., 2007; Kouassi et al., 2007). Ravanini et al. (2007) studied a group of HIV- infected patients living in Italy including 63 symptomatic (A class CDC, eighteen patients on antiretroviral, ARV, therapy) and 8 symptomatic patients (B or

C class CDC, 5 patients on ARV therapy). Haslin and Chermann (2007b) studied 507 HIV-infected (on HAART or not and 201 uninfected individuals from USA. The study done by Tagny et al. (2007) was conducted with a group of HIV-infected individuals naïve of ARV therapy and living in Cameroon including 22 asymptomatic (A1 and A2 class CDC 1993) and 61 symptomatic (A3, B or C class CDC 1993) patients. Lastly, 100 HIV-infected individuals (50 naïve of HAART and 50 on HAART) living in the Ivory Coast were used in the study done by Kouassi et al. (2007). Thirty six patients were at the clinical stage A according to CDC and classified asymptomatic and 53 patients classified symptomatic (CDC stage not stated). None of these four studies report on precipitation assays or any other validation study to confirm that the antibodies detected were R7V antibodies. These four studies are discussed in Table 1 with some additional observations described below. Haslin and Chermann (2007b) observed that R7V antibodies from HIV-1 subtype B infected patients were able to neutralize HIV-1 subtype D and that higher titres of R7V antibody plasma were more effective at neutralizing HIV-1, suggesting that the neutralizing potential most likely was due to R7V antibodies. A positive correlation between R7V antibody ratio and viral load for a group of asymptomatic patients naïve of ARV therapy (n = 45) was observed by Ravanini et al. (2007) and supported by similar observations by Sanchez et al. (2008).

A vaccine component should induce neutralizing antibodies and/or a cellular immune response depending on how it is presented to the immune system. Evidence summarized in this review demonstrates that R7V as antigen in animal studies induced neutralizing antibodies in rabbits (Galéa et al., 1999a). Recombinant MAbs to R7V neutralized viral isolates (Haslin and Chermann, 2004; Haslin et al., 2007a) and naturally produced R7V antibodies isolated from humans did the same (Galéa et al., 1999a and 1999b; Haslin and Chermann, 2007a). In addition, MAbs to β2m, the parent protein of R7V, also neutralized multiple viral strains (Arthur et al., 1992). This evidence supports large scale studies on either protein or peptide for induction of protective humoral immune responses. These studies should perhaps be preceded by studies demonstrating the extent to which β2m or R7V induce autoimmune responses.

R7V antibodies and autoimmunity

A lot still remains to be done with regards to the peptide R7V and what it could mean for prognosis of disease, therapeutics or HIV vaccine development. According to the patent of Chermann et al. (2006) vaccine research has been done with formulations of R7V conjugated to carrier proteins such as KLH and BSA. Because the epitope is host derived one may assume a concern of the possibility that R7V antibodies may initiate an autoimmune response. However, work by Galéa et al. (1996 and 1999a and b), Haslin and Chermann (2002) and Haslin et al. (2007a) suggest this not to be the case. Data are not shown in these papers but according to the discussion there appears to be no self recognition by R7V anti-bodies; no binding to the surface of human cells by purified R7V antibodies from either patients or immunized rabbits (Galéa et al., 1999b). Nor did the recombinant monoclonal R7V antibody made by Haslin et al. (2007a) bind to human cells. In addition, individuals with naturally high levels of R7V antibodies did not exhibit any autoimmune diseases (Galéa et al., 1996 and 1999b). This suggests that R7V antibodies produced inside an individual were virus specific and therefore not a problem to the host. If there are no autoimmune antibodies it could be due to the fact that the R7V peptide is presented to MHC class II (for antibody production) in the context of viral molecules. R7V anti-bodies would therefore not recognize a corresponding epitope on the surface of the host cells. Also, as men-tioned before, the R7V epitope is only visible to the immune system when this epitope is contained in HIV and not in the natural protein.

R7V and cellular immunity

People exposed to HIV mount an immune response which in some individuals slows down disease progression. Strong neutralizing antibodies (Watkins et al., 1996; Pilgrim et al., 1997; Richman et al., 2003; Wei et al., 2003) and strong cytotoxic T-lymphocyte (CTL) responses (Wagner et al., 1999; Cao et al., 1995; Klein et al., 1995) have been detected in LTNPs. This means that immune responses slow down disease progression and is certainly of value for prognosis of disease and vaccine development. An HIV vaccine should presumably induce humoral and cellular responses. Neutralizing antibodies as part of a humoral response should eliminate cell-free virus. Cellular immune responses (e.g. CD8+ CTLs) on the other hand should remove already infected cells that escape antibody-mediated neutralization (Lemckert et al., 2004). If R7V is to be considered in vaccine research, knowledge of possible cellular immune response epitopes within it is of importance. CTL responses are mostly related to viral core proteins (Buseyne et al., 1993; Cao et al., 1997; Nakamura et al., 1997; McAdam et al., 1998), but it has been observed with some viral envelope proteins as well (Pinto et al., 1995). R7V (even though it is derived from a protein associated with and important for MHC class I presentation) is recognized as a foreign antigen by the host and induces a humoral immune response as is evident in all the reports on R7V antibodies. It would be interesting to know whether virus associated R7V-like epitopes are capable of inducing a cellular immune response especially given its β2m origins and the role of this protein in MHC class I presentation of antigen. MHC class I molecules are known to bind epitopes ranging from eight to eleven amino acids (Karim and Karim, 2005) and therefore might

bind the seven amino acid R7V peptide for presentation during natural infection. No HIV incorporated host proteins have yet been implicated in CTL responses nor has this been exhaustively investi-gated.

CONCLUSION

R7V antibodies have been suggested as a potential therapeutic tool (Haslin and Chermann, 2002, 2004 and 2007b; Haslin et al., 2007a) since 2002. To date no studies report on passive infusion of animal models (or human volunteers) with these R7V antibodies. Nor have recombinant R7V antibodies (Haslin and Chermann, 2004; Haslin et al., 2007a) been used in *in vivo* therapeutic studies. Passive infusion of animals (Binley et al., 2000; Mascola et al., 2000 and 2003; Mascola, 2002) or humans (Armbruster et al., 2004) with HIV antibodies is not uncommon. Suggestions of a synthetic R7V peptide being considered as a vaccine has been made since 1996 (Le Contel et al., 1996; Galéa et al., 1999a and b; Chermann, 2001; Haslin and Chermann, 2007b). No studies evaluating the *in vivo* value of R7V antibodies have yet been reported. By this we mean eliciting *in vivo* neutralizing antibodies in animal models (using synthetic R7V as antigen) and challenging this response with live virus. Subsequently the literature also does not report on any R7V based (phase I/II) vaccine trials. It appears that vaccine development studies have been done and some of it has been published as patents (Chermann et al., 2000 and 2006). Because the prevalence of the R7V antibodies was shown to correlate with non-progression to AIDS it has been postulated that patients who have elevated levels of R7V antibodies have a lower likelihood of progression to AIDS. Further studies are needed to clarify the use of R7V antibodies as possible prognostic markers. The presence of these antibodies in uninfected individuals needs to be clarified as well since it has implications for the former statement. Finally, whether the R7V epitope or the entire β2m protein is incorporated by other or all enveloped viruses has implications for the use of the epitope or antibodies to it in prognosis or therapy. Limited sample number studies commenting on possible cross-reactivity between R7V antigen and antibodies from other enveloped viruses exist but needs to be expanded. There are similarities between HIV and HTLV and there are reports of non-random incorporation of host proteins by the latter which further supports clarifying the extent to which β2m or R7V is incorporated by other viruses.

R7V and antibodies to the epitope holds promise but more extensive and clarifying autoimmunity and cross-reactivity studies are needed to maintain optimism on its use. That a host epitope when incorporated in a viral envelope is immunogenic has been sufficiently demonstrated. What remains to be rigorously shown is the usefulness of the immunogenicity (challenging the neutralisation response with live virus) after autoimmunity due to these host antigens have been shown to not be a concern.

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