The Characterization of HIV-1 Specific CD4+ T Helper Epitopes

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Introduction

There is increasing evidence that CD4+ T helper cells play a critical role in the maintenance of virus-specific immunity in most chronic viral infections (Matloubian, Battegay, van Herrrath, Walter). Unfortunately, in the majority of HIV-1 infected individuals, these cellular responses are functionally impaired in all stages of disease (Wahren, Berzofsky, Krowka, Pontesilli, Schrier, Rosenberg). The most notable exception to this > observation is that the robust T helper cell responses are observed in persons with long-term non-progressive infection (Rosenberg). Furthermore, in studies of untreated chronic HIV-1 infection, virus-specific T helper cell responses are inversely correlated with plasma HIV-1 viremia suggesting that these cells provide an important role in viral control (Rosenberg, Kalams). Recently, significant advances have been made in determining the functional relevance of these CD4+ T helper cell responses. For example, several reports from the SIV macaque model indicate that virus-specific T helper cell responses are essential for viral control when therapy is discontinued (Barouch, Hel, Lifson, Lori). The functional relevance of HIV-1 specific T helper cell responses has been further defined by human treatment interruption studies (Lisziewicz, Rosenberg). Finally, vaccine studies have shown that Th1 help is required for a successful cytolytic T lymphocyte (CTL) response (Gahery-Segard, Mortara).

Despite mounting evidence that HIV-specific T helper cells play a critical role in the immune response against HIV-1, little is actually known about the precise epitopes targeted by these cells. A successful immunotherapeutic strategy is likely to require successful induction of HIV-1 specific antibody, CD8+ CTL and CD4+ T helper responses. The identification of a repertoire of dominant HIV-1 specific T helper epitopes that can bind to the most common HLA alleles in the population will be critical to this process (Bangham). The purpose of this review is to provide an overview of those best-characterized human HIV-1 specific T helper cell epitope regions that have been described to date in natural infection.

Characteristics of T helper epitopes

CD4+ T cells are stimulated by peptides presented in association with Major Histocompatibility Complex (MHC) class II. The peptides are derived mainly from exogenous proteins that have undergone proteolytic degradation and processing within the endocytic pathway of antigen presenting cells (APCs) (reviewed in Watts and Geuze). In brief, these antigens bind to APCs and are internalized via intracellular vesicles in which they undergo proteolytic degradation in preparation for loading onto MHC class II molecules. The alpha and beta chains of these molecules are simultaneously synthesized and dimerized within the endoplasmic reticulum (ER) with the C-terminal domains forming the peptide-binding groove. These alpha-beta heterodimers then associate with a membrane glycoprotein, termed the invariant chain (Ii), whose lumenal domain, class II-associated Ii peptide (CLIP) functions to block the peptide-binding groove from any class I peptides within the ER (Roche). This complex is transported to endosomes and delivered to the endocytic pathway to associate with the peptides processed from exogenous antigen. As opposed to class I molecules, the class II binding groove is open and thereby able to accommodate longer peptides of 13–18 amino acids. The majority of identified class II associated peptides have been of this length, although a crystallization model (Reinherz) suggests that a central core of as few as 9 amino acids actually come into contact with the T cell receptor. Confirmation of this report is provided by recent published data (Malhotra) and Norris *et al*. (personal communication) that identify HIV-1 specific T helper epitopes of 9 amino acids in length. Peptides that bind to class II molecules lack conserved anchor residues at the ends of the peptide. Rather, class II molecules and their associated peptide interact via hydrogen bonding along the length of the central peptide (Madden). In general, this sequence is composed of an aromatic or hydrophobic residue at the amino terminus and three additional hydrophobic residues in the central portion and at the carboxyl-terminus. Elution of peptides from class II molecules has shown that over 30% have a proline residue at position two and a cluster of prolines at the carboxyl terminal end (Jardetsky).

Methods for measuring T helper responses

Historically, several *in vitro* assays have been used to detect T helper responses in HIV-1 infection. To date, however, there has been no agreed upon standard methodology for the utilization of these assays or for the minimum responses required to be considered significant. The measurement of cytokine secretion in the supernatant of antigen stimulated cells was the method predominantly used initially. Subsequently, the Lymphocyte Proliferation Assay

(LPA), measuring [3H] thymidine incorporation in counts per minute (CPM) by antigen stimulated cells has been more commonly used. Results are reported as either CPM, delta counts per minute (DCPM: the CPM in the stimulated sample minus the CPM in the negative control), or Stimulation Index (SI: the mean CPM in the stimulated sample divided by the mean CPM in the negative control). This assay is attractive because *in vitro* proliferation is presumed to be correlated with in vivo function. However, methodology with regards to incubation time, concentration of stimulating antigen and cutoffs for minimum SI or DCPM signifying a positive response have varied between studies.

Most recently, newer assays have been utilized which measure cytokine secretion with high sensitivity. The Elispot assay measures cytokine secreted by a single cell though without CD8 depletion it is not immediately able to differentiate between T helper and CTL responses. Further specificity is provided by Intracellular Cytokine Staining (ICS) which quantifies, by flow cytometric gating of CD4 cells, cytokine production by this cell population. As opposed to all of the prior methods it has the immediate advantage of being CD4+ specific. Despite the potentially increased sensitivity of these assays for identifying T helper responses, the correlation with functional capacity remains to be elucidated.

HIV-1 specific T helper epitopes

Multiple laboratories have contributed to the identification of HIV-1 specific T helper epitopes. Although few epitopes have been fully characterized and fine mapped in natural infection, there are multiple promising epitopic regions that have been studied in murine, non-human primate and vaccine models in non-HIV infected individuals. All of these are listed within the LANL Molecular Immunology Database.

The following table includes the identified human epitope regions that have been characterized in natural infection. It is likely that these regions will be further specified to provide HLA restricted epitopes that may be useful in future immunotherapeutic strategies. In some cases we have included examples where several groups have identified overlapping regions. We anticipate that as these epitopes are further characterized and the minimum or optimal sequences are identified some of these regions may well include multiple epitopes while others may be removed from the list. We have also made note of the methods used to detect the responses and the variation in the assay when applicable. In some cases we have omitted epitopes where the exact sequence was not recorded though these may well prove to be significant epitopes when further defined.

We anticipate that as CD4 epitopes are more specifically delineated in the future more stringent criteria will be adopted and the list will be adapted accordingly to include those epitopes that are likely to be most useful in development of immunotherapeutic strategies. For this list to be most useful, included epitopes should ideally meet the following criteria:

1. A T helper cell response detected in natural infection via LPA, Elispot or ICS. Verification through CD8+ depletion, clonal analysis or flow cytometry should be performed for all cases. Ideally lymphoproliferation assay SI should be greater than 5 and/or DCPM> 600. Further study into the correlation between the various assays is still needed.
2. Truncation data with demonstration of the minimal stimulatory epitope.
3. HLA restriction.

Table 1 HIV-1 specific T helper epitopes that have been characterized in natural infection

Protein	AA[1]	HLA	Sequence	SI:[2] 2–5	SI:[2] >5	Cytokine[3]	N	Reference
p17	21–35	DR13.02	LRPGGKKKYKLKHIV	X[6]		IL-2[4]	NA	Harcourt98
	33–47	ND	HIVWASRELERFAVN	X		NT	9/16	Wahren89
	93–107	ND	EIKDTKAEALDKIEEE	X		NT	4/16	Wahren89
	118–132	ND	AAADTGHSSQVSQNY	X		NT	4/16	Wahren89
p24	1–11	DR1	PIVQNLQGQMV	X		IL-2[4]	NA	Harcourt98
	1–15	ND	PIVQNLQGQMVHQAI		X[7]	NT	10/16	Wahren89
	1–22	ND	PIVQNIQGQMVHQAISPRTLNA		X	IFN-γ[5] U	1/2 LTNP	Rosenberg97
	31–52	ND	AFSPEVIPMFSALSEGATPQDL		X	IFN-γ[5] U	2/2 LTNP	Rosenberg97
	48–62	ND	TPQDLNTMLTVGGH		X[8]	NT	8/19	Adams97
	76–85	ND	EAAEWDRVHP		X[8]	NT	11/24	Adams97
	76–90	ND	EAAEWDRVHVHAGP	X		NT	6/16	Wahren89
	81–102	ND	DRVHPVHAGPIAPGQMREPRGS		X	IFN-γ[5] U	1/2 LTNP	Rosenberg97
	96–110	ND	MREPRGSKIAGTTST	X		NT	6/16	Wahren89
	111–132	ND	LQEQIGWMTNNPPIPVGEIYKR		X	IFN-γ[5] U	2/2 LTNP	Rosenberg97
	119–138	DRB1*1302	TNNPPIPVGEIYKRWIILGL		X	IFN-γ[4,5]	NA	Malhotra01
	128–137	DRB1*1302	EIYKRWIILG		X	IFN-γ[4,5]	NA	Malhotra01
	131–152	ND	KRWIILGLNKIVRMYSPTSILD			IFN-γ[5] U	2/2 LTNP	Rosenberg97
	135–154	ND	ILGLNKIVRMYSPTSILDIR		X[8]	NT	8/24	Adams97
	146–160	ND	SPTSILDIRQGPKEP	X		NT	8/16	Wahren89
	156–170	ND	GPKEPFRDYVDRFYK	X		NT	8/16	Wahren89
	156–174	ND	QPKEPFRDYVDRFYKTLRA		X[8]	NT	5/21	Adams97
	163–177	DRB1*1302	DYVDRFYKTLRAEQA		X	IFN-γ[4,5]	NA	Malhotra01
p15	30–44	ND	FNCGKEFHTARNCRA	X		NT	5/16	Wahren89
	55–69	ND	KEGHQMKDCTERQAN	X		NT	5/16	Wahren89
	60–74	ND	MKDCTERQANFLGKI	X		NT	6/16	Wahren89
	98–112	ND	ESFRSGVETTTPPQK	X		NT	8/16	Wahren89

Table 1 cont.

Protein	AA[1]	HLA	Sequence	SI:[2] 2–5	SI:[2] >5	Cytokine[3]	N	Reference
RT	36–52	ND	EICTEMEKEGKISKIGP	NT	NT	IL-2[4]	9/17	Degroot91
vpr	66–80	ND	QLLFIHFRIGCRHSR	X		NT	6/16	Sarobe94
rev	9–23	ND	DEELIRTVRLIKLLY	X		IL-10[4]	NA	Blazevic95
	25–39	ND	SNPPPNPEGTRQARR	X		IL-10[4]	NA	Blazevic95
	33–48	ND	GTRQARRNRRRRWRER	X		IL-10[4]	NA	Blazevic95
	41–56	ND	RRRRWRERQRQIHSIS	X		IL-10[4]	NA	Blazevic95
gp160	37–47	ND	VYYVPVWKEA		X	NT	8/17	Nehete98
	105–117	ND	HEDIISLWDQSLK	NT	NT	IL-2[4]	4/35	Clerici89
	112–141	ND	WDQSLKPCVKLTPLCVSLKCTDLGNATNTN		X V	NT	11/36	Sitz99
	147–168	ND	MMMEKGEIKNCSFNISTSIRGK		X V	NT	19/36	Sitz99
	185–215	ND	NDTTSYTLTSCNTSVITQACPKVSFEPIPI		X V	NT	11/36	Sitz99
	199–211	ND	SVITQACSKVSFE	X		NT	NA	Nehete98
	264–287	ND	SLAEEEVVIRSANFTDNAKTIIVQ		X V	NT	18/36	Sitz99
	269–283	ND	EVVIRSANFTDNAKT	X		NT	6/20	Wahren89
	274–288	ND	SANFTDNAKTIIVQL	X		NT	8/20	Wahren89
	308–322	ND	RIQRGPGRAFVTIGK			IL-2[4]	1/22	Clerici89
	309–323	ND	EQRGPGRAFYVTIGKI	X		NT	6/20	Wahren89
	314–328	ND	GRAFVTIGKIGNMRQ	X		NT	7/20	Wahren89
	314–341	ND	GRAFVTIGKIGNMRQAHCNISRAKWNAT		X V	NT	10/36	Sitz99
	332–354	ND	NISRAKWNATLKQIASKLRREQFG		X V	NT	11/36	Sitz99
	364–378	ND	SSGGKPEIVTHSFNC	X		NT	8/20	Wahren89
	369–383	ND	PEIVTHSFNCGGEFF	X		NT	8/20	Wahren89
	394–408	ND	TWFNSTWSTKGSNNT	X		NT	11/20	Wahren89
	418–436	ND	CRIKQIINMWQGVGGKAMYA	X		NT	NA	Nehete98
	422–437	ND	KQIINMWQEVGKAMYA	NT	NT	IL-2[4]	4/35	Clerici89
	438–460	ND	PISGQIRCSSNITGLLLTRDGGN		X V		14/36	Sitz99
	459–473	ND	GNSNNESEIFRPGGG	X		NT	9/20	Wahren89
	476–490	ND	DMRDNWRSELYKYKV	X		NT	8/20	Wahren89
	484–498	ND	YKYKVVKIEPLGVAP	X		NT	8/20	Wahren89

Table 1 cont.

Protein	AA[1]	HLA	Sequence	SI:[2] 2–5	SI:[2] >5	Cytokine[3]	N	Reference
	519–543	ND	FLGFLGAAGSTMGAASLTLTVQARC	X		NT	NA	Nehete98
	547–561	ND	GIVQQQNNLLRAIEA		X[9]	NT	3/23	Wahren89
	562–576	ND	QQHLLQLTVWGIKQL	X		NT	9/23	Wahren89
	586–597	ND	YLRDQQLLGIWG	X		NT	NA	Nehete98
	593–604	ND	LGIWGCSGKLIC	X		NT	NA	Bell92
	647–664	ND	EESQNQQEKNEKNEQELL	X		NT	6/23	Wahren89
	667–681	ND	ASLWNWFNITNWLWY	X		NT	11/23	Wahren89
	682–696	ND	IKLFIMIVGGLVGLR		X[10]	NT	6/23	Wahren89
	827–841	ND	DRVIEVVQGAYRAIR					Clerici89
	842–856	ND	HIPRRIRQGLERILL	X		NT	9/23	Wahren89

[1] Location in HXB2
[2] Stimulation Index in Lymphocyte Proliferation Assay
[3] Cytokine production detected
[4] in supernatant of stimulated cells
[5] Elispot assay
[6] 72 hour incubation on LPA
[7] 10 of 16 HIV-1 positive subjects responded. Mean SI: 6.0
[8] IL2 added to cell culture with antigen for 8 day incubation
[9] 3 of 20 subjects responded. Mean SI: 6.0 in 6 to 7 day LPAs
[10] 6 of 23 subjects responded. Mean SI: 6.0 in 6 to 7 day LPAs
Abbreviations:
N: fraction of seropositive responders to peptide
ND: not delineated
U: unpublished data
NA: not available
NT: not tested
V: vaccinated serpositive individuals
IL: interleuken
IFN: interferon
LTNP: long-term non-progressor

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