TCR Peptide Therapy in Human Autoimmune Diseases*

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Inflammatory Th1 cells reacting to tissue/myelin derived antigens likely contribute to the pathogenesis of diseases such as multiple sclerosis (MS), rheumatoid arthritis (RA), and psoriasis. One regulatory mechanism that may be useful for treating autoimmune diseases involves an innate second set of Th2 cells specific for portions of the T cell receptor of clonally expanded pathogenic Th1 cells. These Th2 cells are programmed to respond to internally modified V region peptides from the T cell receptor (TCR) that are expressed on the Th1 cell surface in association with major histocompatibility molecules. Once the regulatory Th2 cells are specifically activated, they may inhibit inflammatory Th1 cells through a non-specific bystander mechanism. A variety of strategies have been used by us to identify candidate disease-associated TCR V genes present on pathogenic Th1 cells, including BV5S2, BV6S5, and BV13S1 in MS, BV3, BV14, and BV17 in RA, and BV3 and BV13S1 in psoriasis. TCR peptides corresponding to the mid region of these BV genes were found to be consistently immunogenic in vivo when administered either i.d. in saline or i.m. in incomplete Freund's adjuvant (IFA). In MS patients, repeated injection of low doses of peptides (100–300 μg) significantly boosted the number of TCR-reactive Th2 cells. These activated cells secreted cytokines, including IL-10, that are known to inhibit inflammatory Th1 cells. Cytokine release could also be induced in TCR-reactive Th2 cells by direct cell-cell contact with Th1 cells expressing the target V gene. These findings indicate the potential of regulatory Th2 cells to inhibit not only the target Th1 cells, but also bystander Th1 cells expressing different V genes specific for other autoantigens. TCR peptide vaccines have been used in our studies to treat a total of 171 MS patients (6 trials), 484 RA patients (7 trials), and 177 psoriasis patients (2 trials). Based on this experience in 824 patients with autoimmune diseases, TCR peptide vaccination is safe and well tolerated, and can produce significant clinical improvement in a subset of patients that respond to immunization. TCR peptide vaccination represents a promising approach that is well-suited for treating complex autoimmune diseases.

KEY WORDS: T cell receptor; autoimmune diseases; immunotherapy; regulatory cytokines.

INTRODUCTION

Human autoimmune diseases often involve inflammatory T cells (Th1) directed against tissue-specific antigens. These autoreactive T cells are present in healthy individuals but are functionally silent because of natural regulatory mechanisms that normally limit their inflammatory activity and potential for causing tissue damage. One mechanism that has been implicated in

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regulating autoreactive Th1 cells involves autoantigenic peptide components of the T cell receptor (TCR) expressed by potentially pathogenic Th1 cells (1). Both α and β chains are produced in excess during assembly of functional TCR molecules. The unassembled TCR chains may be partially degraded, internally processed, and presented on the T cell surface in association with self MHC I or II molecules, thus providing potential MHC-restricted targets that can combine specifically with the TCR of the regulatory T cells. The density of the MHC/TCR peptide on the T cell surface would likely depend on the rate of synthesis, the abundance of unassembled chains, and the degree of T cell activation, which in turn would influence expression of MHC I and II molecules. The specificity of the regulatory Th2 cells towards the MHC/TCR complexes presented on Th1 cells would be guided by the types and number of binding sequences in the TCR chains that can form stable complexes with the MHC binding domains. The efficacy of the regulatory process would depend upon the abundance of Th1 cells in the tissues which could be targeted by the Th2 cells. Failure of recognition of TCR

sequences by Th2 cells would result in proliferation and expansion of pathogenic Th1 cells. Immunologic exposure (vaccination) to a peptide closely resembling an autoreactive (potentially pathogenic) TCR fragment should enhance Th2 priming/recognition, and thus help to maintain cytokine (IL-10) regulatory control over Th1-mediated inflammation. In this review, we will discuss the scientific rationale and our clinical experience with TCR peptide therapy in patients with multiple sclerosis, rheumatoid arthritis, and psoriasis vulgaris (see Table I).

Organization of the TCR. The TCR is comprised of paired alpha and beta chains, each composed of about 200 amino acid residues which have variable (V) and constant (C) regions separated by joining (J) and diversity (D - beta chain only) segments (2). There are 26 BV gene and 31 AV gene families containing approximately 65 unique BV alleles and about 50 unique AV alleles, and about 50 AJ, 13 BJ, and 2 BD alleles. An almost limitless level of diversity is generated by random TCR rearrangements formed by juxtaposition of almost all segments for each chain. The antigen combining site for each chain is a solvent exposed loop that

Table I. Summary of Clinical Studies Using TCR Peptides

Disease	Designation/Location	Dates	# Pts	Goal
Multiple Sclerosis	Phase I, Oregon	1991–1993	11	Safety & immunogenicity, BV5S2 & BV6S1 CDR2 peptides; effects on MBP response
	Phase I, Oregon	1994–1996	11	Safety & immunogenicity, overlapping BV5S2 peptides, BV3, BV9, BV12S2
	Double blind phase I/II, Oregon	1994–1995	23	Safety & immunogenicity, BV5S2 CDR2 peptides; effects on MBP response
	Double blind phase I/II Multicenter (Connetics)	1996–1998	106	Safety & immunogenicity, BV5S2 CDR2 peptides; effects on MBP response
	Phase I, San Diego (IRC)	1994	10	Safety & immunogenicity, BV6S5 CDR2 peptide; effects on CSF cellularity
	Phase I, Multicenter (IRC)	1997–1999	10	Safety & immunogenicity, BV6S5 CDR2 peptide; responses in unselected patients
	Double blind phase II, Multicenter (IRC)	2001–2002	60	Safety & immunogenicity, [Y49T]BV5S2, BV6S5, BV13S1 CDR2 peptide cocktail, MRI
Rheumatoid Arthritis	Phase I, Alabama, San Diego (IRC)	1992–1994	15	Safety & immunogenicity, BV17 CDR2 peptide, dosing, effects on activated T cells
	Phase I, Alabama, San Diego (IRC)	1994–1995	13	Safety, immunogenicity, dosing of BV17 CDR2 peptide
	Phase I, Alabama, San Diego (IRC)	1993–1994	17	Safety, immunogenicity, dosing of BV14 CDR2 peptide
	Double blind phase II, Multicenter (IRC)	1995–1996	99	Safety, immunogenicity, ACR20 scores, dosing of BV17, BV14, BV3 CDR2 peptide cocktail
	Double blind phase IIb, Multicenter (IRC)	1997–1998	340	Safety, immunogenicity, ACR20 scores, dosing of BV17, BV14, BV3 CDR2 peptide cocktail
Psoriasis Vulgaris	Double blind phase II, Multicenter (IRC)	1995–1996	93	Safety, immunogenicity, lesion & PASI scores, dosing of BV3 & BV13S1 CDR2 peptide cocktail
	Double blind phase II, Multicenter (IRC)	1997–1998	84	Safety, immunogenicity, lesion & PASI scores, dosing, carriers, of BV3 & BV13S1 20 mer vs 40 mer peptide cocktail

contains a unique peptide sequence (complementarity determining region 3, CDR3) formed by the insertion of additional nucleotides (N region additions) into the sequence spanning the BV-BD-BJ or AV-AJ junctions. All combinations of TCR genes allow for approximately 10^{15} unique TCR specificities (3).

Natural T-T Regulatory Circuits. T cell recognition of self TCR sequences represents an effective autoregulatory mechanism for limiting inflammatory reactions mediated by Th1 cells such as those directed at organ-specific antigens (4–6). This mechanism, as currently postulated, involves the display of internally processed TCR determinants in association with MHC class I and II molecules on the surface of Ag-specific T cells, a complex that can both stimulate and function as a target for anti-TCR-specific T cells (7,8). The biological outcome of T-T interactions directed at TCR epitopes depends on the functional properties of the responsive T cells and the sensitivities of the stimulator/ target T cells. Thus, CD4+ T helper cells recognize a variety of specific TCR determinants on AV and BV chains in association with MHC class II molecules, primarily HLA-DR (9), and in unprimed individuals these T cells have been shown to secrete both IFN-γ and IL-10, but not IL-4, suggesting they are of the Th1/Th0 subtype (10). However, in patients successfully vaccinated with TCR peptides, the TCR-reactive T cells produced both IL-4 and IL-10 but little if any IFN-γ, indicating a Th2 subtype (8). TCR-reactive CD4+ T cells from unprimed or vaccinated individuals were not cytotoxic for target Th1 cells expressing the cognate TCR unless the targets were prepulsed with the respective TCR peptide (11,12). However, T-T interaction did induce proliferation and cytokine responses by the TCR-specific T cells (Chou et al., in preparation). These important findings demonstrate the capacity of intact Th1 cells to directly activate TCR-specific Th2 cells, strongly implying display of internally processed TCR molecules.

The release of Th2 cytokines, especially IL-10, by TCR-reactive Th2 cells inhibited proliferation and IFN-γ production by the stimulating Th1 cells (8,9). IL-10 has potent inhibitory properties on inflammatory T cells (13), and can prevent induction of EAE (14,15). Presumably, local release of IL-10 by TCR-specific T cells within the tissue could inhibit not only the stimulating Th1 cell bearing the cognate TCR, but also bystander Th1 cells, including those expressing distinct V genes specific for other antigens. Such a mechanism could also down-regulate production of proinflammatory cytokines such as TNF-α by monocytes and macrophages. Thus, autoimmune inflammatory infiltrates consisting of tissue Ag-specific as well as recruited Th1 cells, as well as other inflammatory cell types, could be down-regulated by TCR-reactive Th2 cells directed at only a single represented TCR (16). Systemic activation of TCR reactive Th2 cells might also occur in response to activated HLA-DR+ Th1 cells within the circulation, but inhibitory effects on the Th1 cells would likely be minimized by rapid dilution of the secreted cytokines within the vasculature.

In addition to regulatory CD4+ T cells, cytotoxic CD8+ T cells could respond to MHC I-associated TCR determinants present on target Th1 or Th2 cells (17,18), or to APC after processing of shed TCR molecules. Induction of CD8+ T cells specific for clonotypic or ergotypic determinants on Th1 cells has been demonstrated after whole cell vaccination in MS patients (19), resulting in a relatively long-term deletion of selected myelin-reactive Th1 cell populations (20). Moreover, cytotoxic CD8+ T cells can be induced in vitro to TCR determinants by autologous, attenuated CD4+ T cell lines or clones (21). In both of these studies, the target TCR determinants were not mapped, precluding the use of defined peptides to boost the regulatory T cells. Generally, this approach appears to be highly effective for inhibiting selected autoreactive T cell clonotypes, but requires individual preparation of large quantities of T cell clones for vaccination.

TCR As Antigen. The most selective regulation mediated by anti-TCR T cells would be directed at the CDR3 sequences that define the clonotype of the pathogenic T cell. In autoimmune diseases, there are many possible pathogenic clonotypes induced to several immunodominant peptides present in multiple tissue antigen proteins. Thus, successful regulation directed at CDR3 motifs would likely require many regulatory T cell specificities, unless pathogenic responses were restricted to just a few dominant clonotypes, an event that does occur occasionally in some patients. A further complication is that not all CDR3 sequences, which range from 8–15 amino acids, contain relevant MHC-binding motifs, and thus may not be immunogenic. Nevertheless, clonotypic regulation has been demonstrated elegantly using anti-sense peptides (22), or after whole cell vaccination using attenuated pathogenic clones (19).

A less selective and therefore a more available target for regulation is the set of TCR AV and BV regions (23). As mentioned above, the total pool of V genes is about 115 unique sequences, although many of these proteins are pseudogenes or orphan genes that may be rarely expressed. It is known that responses to autoantigens often involve oligoclonal expansions of T cells that express the same or similar V genes, despite

having distinct specificities for different epitopes on the same autoantigen (24). These observations suggest diminished V gene specific regulation that would allow selective expansion of different TCR clonotypes sharing common V genes. Although most families of V genes have been implicated in T cell responses to autoantigens, there are clearly some V genes that are utilized more often than others (25). Conceptually, this characteristic could allow targeting of a limited set of V genes that likely would be represented in the autoreactive T cell pool in most patients. Although these widely shared sets of V genes have not yet been fully defined for the key autoimmune diseases, there are nevertheless many reports that implicate certain over-expressed V regions that are useful starting points for developing therapeutic vaccines. Moreover, it is likely that a cocktail of V region peptides will be required to obtain a wide enough base of immunization to regulate a critical percentage of pathogenic T cell clones in a substantial number of diseased patients.

TCR Studies in Multiple Sclerosis (MS)

MS May Involve Myelin Reactive T Cells. MS is a chronic disease of the central nervous system (CNS) that is characterized by progressive loss of motor and sensory nerve function and immune-mediated inflammation and demyelination (26). Although the cause of MS is unknown, it is probable that Th1 cells specific for encephalitogenic myelin antigens, including myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) contribute to its pathogenesis. Collectively, data supporting a role for myelin antigens in the pathogenesis of MS include: 1) increased frequency of MBP-, PLP-, MOG-, and α-B-crystallin-specific T cells in the blood or cerebrospinal fluid (CSF) of MS versus control patients (27–34); 2) increased state of activation of MBP- and PLP-specific, or other oligoclonal T cells in the blood and CSF of MS patients (29,35–39); 3) sporadic increases in the frequency of MBP-specific T cells that correlate with disease activity in at least half of MS patients (8,40); 4) possible therapeutic efficacy of intervention strategies directed at MBP-specific T cells (8,19,41); 5) worsening of MS in some patients treated with an altered peptide ligand of MBP-85-99 (42); and 6) demonstrated encephalitogenicity of MBP, PLP, and MOG, and transfer of experimental autoimmune encephalomyelitis (EAE) with myelin-specific T cells in animal models representing a wide variety of MHC backgrounds (43–50). Because of the extensive litera-

ture available, and the recently reported ability of MBP-specific clones to be stimulated by a variety of microbial and self antigens (51), we regard MBP as the leading prototypic myelin target antigen in MS, but recognize that PLP, MOG, or other as-yet-unrecognized myelin antigens may also participate as encephalitogens.

In MS patients of northern European descent, there is a disease association with HLA-DR2 and -DQw6, with different class II associations (i.e. DR3, DR4, and DR6) in other populations (52–55). These MHC molecules could contribute to disease susceptibility, although their exact role in the MS disease process has not been defined mechanistically. It is possible that class II molecules may present encephalitogenic myelin epitopes to pathogenic T cells, or conversely, these molecules may be inefficient at presenting regulatory peptides (i.e. TCR peptides) to regulatory T cells that normally limit the activation of autoreactive T cells.

Preferential Utilization of TCR V Region Genes Provides a Target for Immunoregulation of MBP-Specific Th1 Cells. In certain rodent models of MS, pernicious T cells specific for MBP preferentially utilize AV2 and BV8S2 genes in their TCR (56). As discussed above, we and others developed TCR peptide and recombinant vaccines corresponding to the BV8S2 sequence that could induce regulatory T cells and antibodies that prevented or reversed clinical paralysis in EAE (6,57–61). Moreover, others have induced protection against collagen induced arthritis with a recombinant TCRAV11 protein (62), indicating that AV chain determinants may also function as targets for immune regulation. In these animal models, peptides from CDR1, CDR2, FW3, and CDR3 all had protective activity against autoimmune disease (6,63–65). Of these, CDR1, CDR2, and FW3 determinants are V gene specific, whereas CDR3 peptides would likely be clonotypic. Because of the possibility that anti-TCR responses can affect bystander Th1 cells, we believe that the broader scope of regulation triggered by V gene determinants would be advantageous over regulation directed at relatively rare CDR3 determinants characteristic of single clones.

MBP-specific T cells from MS patients are often oligoclonal and tend to over-utilize certain V region genes, although the pattern of expression in humans is obviously much more diverse than for inbred rodents (66). Thus, although a number of different V genes have been reported to be over-expressed in MS patients, it is likely that predominant disease-relevant V genes are different among individuals (67). Nevertheless, a few V genes have been associated with MS in several studies using quite different approaches. Our initial studies

showed a remarkable bias in the use of BV5S2, and to a lesser extent BV6 and AV8, by MS T cells specific for a variety of MBP determinants (24). Concurrently, others found preferential expression of BV5 genes by T cells from DR2+ MS CNS plaque tissue (68), and many of the CDR3 sequences were similar to MBP-specific T cell clones, suggesting that BV5 TCRs might be relevant to the pathogenesis of MS. Additional studies found a selective marked expansion of CD4+ BV5S3+ T cells in recently diagnosed DR2+ MS patients (69), and BV5S2+ T cells with MBP CDR3 sequences in MS CSF and brain biopsies (70). Other studies, however, have not observed increased expression of BV5 by MBP-specific clones (71) suggest regional sampling differences. In seeking further evidence of restricted V gene usage in MS, we recently reviewed more than 20 reports in the literature describing V gene use by >600 MBP-specific T cell isolates (25). We concluded that two of the V genes implicated by individual studies, AV8 and BV5, were used more frequently by MBP-specific T cells from MS patients than by controls. The increased use of AV8 and BV5 did not differ between DR2+ and DR2− patients, suggesting that this MHC class II molecule that is also MS disease associated did not contribute to the selection of AV8 or BV5 genes. Conceivably, there may also be other MS-associated V genes. These striking observations validate the concept that certain V genes may indeed be utilized by a significant percentage of MS patients in response to a widely encephalitogenic molecule (MBP).

MS May Involve Defective Immunoregulatory Control. Several lines of evidence lead to the possibility that the autoimmune attack in MS is facilitated by a loss of immunoregulation. In a series of studies by Antel and colleagues, defective regulation was observed in relapsing and progressive MS patients (72,73). Much of the missing suppressor activity in MS patients was in the CD8+ cell subpopulation (74,75), although no differences were noted in cytotoxicity effector functions. This defect in immunoregulation was significantly more pronounced in MS patients with active or progressive disease than in those with stable disease (76), suggesting a role of these regulatory cells in controlling disease activity. Our recent studies further suggest that *CD4+ Th2* regulatory cells directed at TCR determinants are also decreased or absent in many MS patients (77).

A likely mechanistic model of MS is that the ongoing disease process drives an oligoclonal expansion of myelin-reactive Th1 cells, which in the absence of effective regulatory anti-TCR responsive T cells can be activated to produce CNS inflammation and damage. If key anti-TCR specificities are lost or depleted, these un-regulated Th1 cells to myelin or other stimuli could now expand, resulting in an over-expression of the unregulated V genes. This working hypothesis would provide a clear explanation of the V gene bias involving many T cell specificities in response to MBP that has been observed in some MS patients (24). It is conceivable that the regulatory defect is selective for certain V genes, thus allowing expansion of only certain clonotypes that would normally be controlled by the corresponding anti-TCR specificities. Conceivably, patients at risk of developing MS or those with other autoimmune diseases characterized by oligoclonal expansion could have a similar regulatory defect.

Clinical Studies in MS with TCR Peptide Vaccines. TCR peptide vaccines have been utilized to induce regulatory T cells in a total of 171 MS patients, with some apparent clinical impact. Our studies in MS initially focused primarily on BV5S2, which was over-represented on MBP-specific Th1 cells from MS patients but not controls (24). Repeated intradermal injection of low doses (100–300 µg) of a Y49T substituted BV5S2-38-58 peptide induced significant T cell responses in about half of the progressive MS patients treated in three separate studies: an open label study (Table II and ref. (78) a follow-up double-blind, placebo controlled pilot trial (Table III and ref. (8); and a multi-center placebo-controlled trial involving 106 MS patients (Table IV). Interestingly, there was an inverse correlation of immune responsiveness to the BV5S2 peptide and responsiveness to MBP, suggesting a regulatory effect of TCR specific T cells on MBP-reactive T cells (Table III). Finally, when our study patients were evaluated collectively for clinical changes, we found a highly significant ($p < 0.001$) correlation between the degree of response to the vaccinating peptide and lack of progression of MS (79). Remarkably, a few patients who had very strong T cell responses to the vaccinating peptide exhibited clinical *improvement,* suggesting that high frequencies of regulatory T cells had the potential to at least partially reverse clinical deficits.

The basic findings from the smaller pilot trials carried out in Oregon were for the most part substantiated by the larger multi-center trial throughout the USA (Table V), especially the enhanced immunogenicity of the [Y49T]BV5S2 CDR2 peptide versus the native sequence. Of note, however, was the observation that the degree of T cell response to injected TCR peptides observed in the multi-center trial was markedly reduced compared to the local trials in Oregon. This reduced sensitivity probably resulted from the need to ship fresh blood samples overnight across the country for analysis >24 hrs after sample collection. We have observed a

Table II. Phase I Open Label Study with BV5S2 and BV6S1 Peptides in Relapsing or Secondary Progressive MS (12,106)

Study Design

-Treatment regimen:
  Eleven patients with RR or SP MS
  Four weekly i.d. injections, then once/month for up to 16 months
  Doses started at 100 μg, increased up to 3000 μg
  [Y49T]BV5S2-38-58 and [G41L]BV6S1-38-58 peptides in saline
  Patients were started on one peptide and second peptide added
-Monitoring
  T cell and antibody responses to TCR peptides, monthly
  T cell responses to MBP and mitogens, monthly
  Clinical (ambulation, upper extremity), 3 monthly for up to 16 months

Study Results

-Safety
  Injections were safe and well tolerated
  One biopsy proven case of leukoclastic vasculitis at 3000 μg dose
-Immunologic responses
  No changes in responses to mitogens, recall antigens or general immunity
  Significant increase in T cell frequency to [Y49T]BV5S2 in 7 patients
  Significant increase in T cell frequency to BV6S1 peptide in 6 of same 7 patients
  Concomitant decrease in T cell frequency to MBP in some patients
  Doses of peptide >300 μg inhibited α-TCR T cell responses
  TCR reactive clones produced IFN-γ, IL-4, and IL-5
-Clinical responses
  Of the 7 immunological responders, 2 improved, 2 were stable, and 3 were worse at end of study.
  All 4 immunological non-responders were worse at end of study

Conclusions

-Both BV5S2 and BV6S1 CDR2 peptides were immunogenic in >50% of patients
-Optimal dose of peptide was between 100–300 μg per injection
-Anti-TCR peptide reactive T cells secrete Th2 cytokines
-No anti-peptide antibodies observed
-No general immunosuppression observed
-Peptide administered i.d. in saline appeared to be safe and well tolerated

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Table III. Open Label Study of Overlapping BV5S2 Peptides, BV3, BV9, and BV12S2 Peptides in Secondary Progressive MS Patients (79)

Study Design

-Treatment regimen:
  11 SP MS patients
  Four weekly i.d. injections, then once/month for two additional months
  Doses of 100–200 μg peptide per injection
  Overlapping 20 mers of BV5S2, BV3-38-58, BV9-38-58, BV12S2-38-58
-Monitoring
  T cell frequencies to TCR peptides prior to each injection and end of study

Study Results

-Safety
  Acute, self-limiting inflammatory reactions at injection site with 4 non-CDR2 peptides
  Otherwise, peptide injections appeared to be safe and well tolerated
-Immunologic responses
  Vaccinating activity localized to core 44–52 determinant of BV5S2
  BV5S2-25-42 only other immunogenic BV5S2 peptide
  Non-responders to BV5S2 responded to CDR2 peptides from other BV genes

Conclusions

-CDR2 peptides were immunodominant
-Patients unresponsive to BV5S2 could be immunized with other relevant BV gene peptides

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60–90% reduction in responses to recall antigens in blood samples processed more than 6 hrs after collection, and postulate that the less robust T cell responses observed in the multi-center trial resulted from sub-optimal conditions. This factor influenced the design of our current trial, in which blood samples from regional MS clinics in the Northwest (Seattle, Tacoma, and Olympia Washington; and Portland and Eugene Oregon) can be shipped to and processed by our laboratory in Oregon within 4–6 hours after collection.

In a separate investigation, Wilson and Gold vaccinated ten MS patients previously screened for increased expression of activated BV6 T cells within the CSF (Table V and ref. (36) (80) with a BV6S5 CDR2 peptide emulsified in IFA. The results indicated that vaccination induced significant immunological responses to the injected BV6S5 peptide, reduced CSF cellularity, inhibited outgrowth of activated CD4+ T cells, reduced expression of BV6 mRNA, and constrained persistence of dominant clonotypes (80). Thus, targeting dominant TCRs from CSF resulted in a marked alteration in the expressed TCR repertoire of cells likely to be involved in the pathogenesis of MS. Of importance mechanistically, inhibition of the outgrowth of all activated T cells in some vaccinated patients strongly supports the notion of bystander suppression. In a follow up study, the BV6S5 CDR2 peptide in IFA was found to be highly immunogenic, even in MS patients who were not pre-screened for expression of BV6S5 by CSF cells (Table VI).

Recently our laboratory has characterized the BV gene expression in almost 160 MBP-specific T cell

Table IV. Randomized Double Blind Placebo Controlled Phase I/II Trial Comparing Native versus [Y49T]BV5S2-38-58 Peptides in DR2+ Primary or Secondary Progressive MS Patients (1,9)

Study Design

-Treatment regimen

23 patients, native or [Y49T] substituted BV5S2-38-58 peptide, placebo

4 weekly i.d. injections, 100 μg/patient, then monthly for 10 months

-Monitoring over 48 weeks

T cell responses to TCR peptides and MBP, monthly

Clinical (ambulation, upper extremity), 3 monthly for 12 months

Study Results

-Safety

Injections appeared to be safe and well tolerated

-Immunologic responses

Significant increase in T cell frequency to [Y49T]BV5S2 in 5/9 patients

Significant increase in T cell frequency to native BV5S2 in 1/8 patients

No increase in T cell frequency to TCR peptides in placebos

Responses to TCR peptide and MBP correlated inversely

TCR-reactive T cells from immunized donors produced IL-4 and IL-10

-Clinical responses

No clinical progression in TCR peptide responders

Response to MBP correlated with clinical worsening

Conclusions

-About 50% of patients responded to vaccination with [Y49T] BV5S2 peptide

-Y49T substituted peptide more immunogenic than native sequence

-Positive correlation between response to peptide and clinical outcome

-Inverse correlation between response to peptide and response to MBP

-Inverse correlation between response to MBP and clinical outcome in subset of patients

Table V. Randomized Double Blind Placebo Controlled Phase I/II Multicenter Trial Comparing Native versus [Y49T]BV5S2-38-58 Peptides in Relapsing and Secondary Progressive MS Patients (in Preparation)

Study Design

-Treatment regimen

106 MS patients, native or [Y49T] substituted BV5S2-38-58 peptide, placebo

4 weekly i.d. injections, 100 or 300 μg/patient, then monthly for 2 months

-Monitoring over 24 weeks

T cell responses to TCR peptides and MBP, monthly

Clinical (ambulation, upper extremity), every 3 months for 6 months

Study Results

-Safety

TCR peptide immunization appeared to be safe and well tolerated

Adverse events were mild, with no differences observed between treated and placebo groups

-Immunologic responses

Patients developed moderate responses to peptide

Higher percentage response to substituted peptide

Higher percentage response to 300 μg dose

No significant difference in peptide response in DR2(+) versus DR2(−) patients

-Clinical responses

No significant disease worsening in peptide-treated versus placebo-treated patients

No correlation between immune response and disease worsening

Conclusions

-About 50% of patients responded to vaccination with [Y49T] BV5S2 peptide

-Y49T substituted peptide more immunogenic than native sequence

-Higher dose of peptide more immunogenic than lower dose

-Response to peptide not related to presence of DR2 allele

clones from MS patients. Evaluation of the rank order of expression of BV genes according to total number of clones indicates BV5 > BV6 > BV13, etc. Similarly, the rank order of expression according to the number of patients expressing a given BV gene family was BV13 > BV5 > BV6. These findings coupled with recent data from Hong et al. (81), identify BV13 as another important BV gene utilized in response to MBP. Based on these findings, we have initiated another clinical trial in MS using a cocktail of [Y49T]BV5S2, BV6S5, and BV13S1 peptides to immunize patients (Table VII). This 60 patient phase I/II study will compare the formulation and route of vaccine administration (peptides/IFA, i.m. versus peptides/saline, i.d.), on immunogenicity, and will evaluate both immune re-

sponses and MRI changes in relapsing-remitting or secondary progressive MS patients.

Overall, our combined clinical data in MS demonstrate that injections of TCR peptide vaccines are safe and well tolerated, with the major adverse event reported being minor injection site reactions. CDR2 peptides were the most immunogenic, although the possibility remains that other regions may also be immunologically and clinically active. The [Y49T]BV5S2 CDR2 peptide has consistently induced significant T cell responses in about half of the patients injected, and the BV6S5 peptide appeared to be even more active, inducing responses in >90% of patients. The BV13S1 peptide has been used previously in psoriasis (see below), and appeared to be immunogenic in about 35% of these patients, although

Table VI. Phase I Trial Using BV6S5 CDR2 Peptide
in MS Patients Pre-Screened for Activated BV6S5+ T Cells
in CSF (36,80)

Study Design
-Treatment regimen
  10 prescreened BV6+ MS patients, BV6S5-39-58 peptide
  Two i.m. injections, 100 or 300 μg peptide/IFA 4 weeks apart,
    5 patients/dose
-Monitoring over 24 weeks
  T cell proliferation responses and antibodies to BV6S5 peptide
  Delayed type hypersensitivity (DTH) responses to peptide
  Effects on BV6+ T cells in CSF
  Clinical (EDSS, Activities of Daily Living) every month for
    6 months
  MRI scans performed before and 24 weeks post-vaccination

Study Results
-Safety
  TCR peptide immunization appeared to be safe and well
    tolerated
  All peptide related adverse events (6/35) were mild injection site
    reactions
-Immunologic responses
  10/10 patients had lymphocyte proliferation response to peptide
    ≥ 2 weeks
  4/5 patients in each dosage group developed DTH responses to
    peptide
  No serum antibody detected to peptide
  Decreased levels of BV6S5+ and other T cells in CSF (5/5,
    300 μg; 1/5, 100 μg)
-Clinical responses
  EDSS and ADL scores remained stable over 24 weeks
  MRI plaque burden decreased (1/5, 300 μg; 2/5, 100 μg)
  MRI active lesions (1/5 decreased, 300 μg; 1/5 decreased, 1/5
    increased, 100 μg)

Conclusions
-BV6S5 peptide in IFA was immunogenic in patients expressing
  BV6S5 in CSF
-TCR peptide therapy inhibited both cognate and bystander
  activated T cells in CSF

Table VII. Open Label Phase I Trial Using BV6S5 CDR2
Peptide in MS Patients Not Pre-Screened for Activated
BV6S5+ T Cells (in Press)

Study Design
-Treatment regimen
  10 patients, BV6S5-39-58 peptide
  Five i.m. injections, 300 μg peptide/IFA, weeks 0, 4, 12, 24, 36
-Monitoring over 48 weeks
  T cell proliferation responses to BV6S5 peptide
  Delayed type hypersensitivity (DTH) responses to peptide
  Clinical (EDSS, Activities of Daily Living) at each visit

Study Results
-Safety
  TCR peptide immunization appeared to be safe and well
    tolerated
  All peptide related adverse events (2/11) were mild injection site
    reactions
-Immunologic responses
  9/10 patients had lymphocyte proliferation response to peptide
    ≥ 2 weeks
  8/10 patients developed DTH responses to peptide
-Clinical responses
  EDSS and ADL scores remained stable over 48 weeks

Conclusions
-BV6S5 CDR2 peptide was immunogenic, even in unscreened
  MS patients

TCR Studies in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disease that results in chronic inflammation and synovial hyperplasia in joint tissue (82,83). There is now considerable evidence that CD4+ T cells may be important in the pathogenesis of RA, based on an association of disease susceptibility and severity with HLA-DR alleles (84,85) and the prominent infiltration of activated CD4+ T cells into synovial tissue (86–88). These synovial T cells normally over-express only a limited number of BV genes, although there apparently are variations from patient to patient.

The pattern of abnormally expressed BV genes in RA patients initially suggested that a microbial super-antigen might be involved in triggering a subset of circulating T cells, including those with TCRs specific for synovial antigens (89,90). Superantigens stimulate T cells non-specifically by bridging selected TCR BV chains and MHC Class II molecules outside the peptide binding groove (91,92). Chronic superantigen stimulation can exhaust the responsive T cells, eventually leading to their reduction in the periphery. However, initial systemic activation of autoreactive T cells could result in their migration into the joint, where further exposure to self antigens might allow local expansion, recruit-

no clinical data are yet available for MS patients. Response to TCR peptide vaccination induced regulatory effects in vivo, as was demonstrated by decreased responses to MBP in patients vaccinated with BV5S2 peptides, and by decreased outgrowth of BV6+ and other CSF clones in patients vaccinated with BV6S5 CDR2 peptide. Perhaps the most important finding from our MS studies is the significant correlation in a subset of patients between the strength of T cell response to immunization and clinical benefit. Some patients with strong immune responses demonstrated clinical improvement, indicating that the TCR peptide vaccination approach under optimal circumstances can have significant therapeutic impact on the clinical progression of MS.

Table VIII. Phase I/II Trial Comparing Immunogenicity of (Y49T)BV5S2, BV6S5, and BV13S1 CDR2 Peptide Cocktail Given in Saline or IFA (in Progress)

Study Design
-Randomized, double blind multicenter trial of 60 RR or SP MS patients
Patients screened 2× by MRI for presence of lesion
25 patients receive peptides in saline, 25 receive peptide/IFA, and 10 receive IFA alone
-Treatment regimen
i.d. injection of 100 μg of each peptide in saline, 4× weekly, 4× monthly
i.m. injection of 100 μg of each peptide in IFA, monthly injections × 3 months
-Monitoring over 24 weeks
Safety of combination peptide cocktail in saline and IFA
Frequencies of proliferating PBMC to each peptide and cocktail (LDAs)
Frequencies of IFN-γ & IL-10 secreting cells to each peptide and cocktail (ELISPOT)
Clinical and MRI changes

ment of additional inflammatory T cells and macrophages, and eventual joint destruction. An alternative mechanism would propose that the RA-associated HLA-DR alleles might preferentially present arthritogenic antigens to CD4+ T cells, thereby propagating synovial inflammation and tissue damage.

Immunotherapies directed non-specifically at T cells generally have not been as effective in RA as treatments directed at neutralizing pro-inflammatory cytokines produced within the joints, notably tumor necrosis factor alpha (TNF-α) and IL-1 (93). However, another more specific immunotherapeutic approach is to target directly the inflammatory T cells that initiate and propagate damage in the synovium. The most difficult question that has not yet been fully addressed is whether the arthritogenic T cells utilize a limited set of V genes that might serve as a target for TCR directed therapies, including vaccination with TCR peptides. This question is complicated by the lack of known human target antigens, although collagen and heat shock protein from Mycobacterium are known to be arthritogenic in rodents.

Many studies have been carried out on V gene expression in synovial tissue and synovial fluid, but there is still little consensus (94). It is widely acknowledged that BV content in the PBMC's is not reflective of what occurs in the synovium. As would be expected in any inflammatory condition involving recruited T cells, the represented TCR repertoire in joints is very heterogeneous and overall is not dominated by particular BV genes (95). Moreover, there is evidence of clonal expansions within the joints of a variety of different V genes. However, activated (IL-2R+) CD4+ T cells from synovial tissue were found to express BV17, BV14, and BV3 at levels significantly higher than other BV gene families (90,96). Although it is still uncertain if these T cells are directed at disease-relevant antigens, their presence in inflamed synovium could provide potential target TCRs for peptide vaccination studies. Conceivably, expression of these BV genes by a substantial proportion of inflammatory T cells in the joint could activate regulatory T cells specific for cognate TCR peptides. Subsequent release of anti-inflammatory cytokines could then regulate not only the target T cells but also bystander cells, including the core arthritogenic specificities that may express either the targeted or different BV genes, as well as other inflammatory cells such as macrophages and neutrophils. Indeed, the major regulatory factor produced by TCR-reactive T cells in MS studies was IL-10 (8), which is known to inhibit both TNF-α and IFN-γ, and has shown some positive clinical effects in RA. Assuming that T cells responding to BV3, BV14, and BV17 can migrate into joint tissue and secrete high levels of IL-10 (or other inhibitory cytokines such as IL-13) in this inflammatory environment, vaccination with the cognate CDR2 peptides could produce beneficial regulation in RA patients.

Based on the over-expression of BV17, BV14, and BV3 on activated T cells from synovial tissue, five different RA trials using TCR peptides in IFA have been carried out using one or more of these TCR peptides in a total of 484 RA patients (Tables I and IX–XIII). Initial studies evaluated the safety and immunogenicity of different doses of BV17 (97) or BV14 CDR2 peptides (Tables IX–XI). These studies demonstrated that about 35% of immunized patients had measurable T cell proliferation responses to injected peptides after immunization, but showed no evidence of antibody responses. Moreover, there were decreased (>20%) circulating levels of activated BV17+ T cells in 6 of 9 patients tested after immunization with BV17 CDR2 peptide (Table IX) (97). Clinically, the TCR treatment resulted in decreased pain and swollen joint scores and an improvement of total joint scores, with effects lasting from 16–44 weeks after the last booster injection of peptide vaccine. In these early phase I open label studies, lower doses of peptide (30–100 μg) appeared to be more active clinically than the higher dose of 300 μg peptide. These studies also showed peptide injections in IFA to be safe, with no indications of significant adverse events.

Based on the promising results and safely profiles, these early studies were followed by two larger phase II trials using different doses of a combination

Table IX. Two Center Phase I Trial of Safety and Immunogenicity of BV17 CDR2 Peptide in Patients with Rheumatoid Arthritis (97)

Study Design

-Treatment regimen
  15 RA patients, BV17 CDR2 peptide
  Two i.m. injections, 10, 30, 100, or 300 μg peptide/IFA
    one month apart
-Monitoring over 48 weeks
  T cell proliferation responses and antibodies to peptide
  Total and activated (CD25+) BV17+ T cells in PBMC
  Clinical (joint pain and swelling scores)

Study Results

-Safety
  TCR peptide immunization appeared to be safe and well
    tolerated
  All peptide related adverse events (4/57) were mild injection site
    reactions
-Immunological responses
  35% of injected patients had increased proliferation responses to
    BV17 peptide
  No detectable antibody to BV17
  Circulating levels of BV17+ T cells were unchanged
  Decreased levels (>20%) of activated BV17+ T cells in 6 of
    9 patients tested
-Clinical responses
  Decreased pain and swelling joint scores at week 48 in all
    groups
  Consistent improvement of total joint scores at all time points in
    all groups

Conclusions

-About 35% of RA patients had immune response after vaccination
 with BV17 CDR2 peptide
-Vaccination reduced levels of circulating activated BV17+
 T cells
-Apparent clinical effect of vaccination at all doses of peptide,
 lasting 44 weeks

Table X. Two Center Phase I Trial of Safety and Immunogenicity of BV17 CDR2 Peptide in Patients with Rheumatoid Arthritis

Study Design

-Treatment regimen
  13 RA patients, BV17 CDR2 peptide
  Three i.m. injections, 30, 100, or 300 μg peptide/IFA one month
    apart
-Monitoring over 24 weeks
  T cell proliferation responses and antibodies to peptide
  Clinical (joint pain and swelling scores)

Study Results

-Safety
  TCR peptide immunization appeared to be safe and well
    tolerated
  One of 7 total adverse events (pruritus) was peptide related
-Immunological responses
  46% of injected patients had increased proliferation responses to
    BV17 peptide
  No detectable antibody to BV17
-Clinical responses
  Decreased pain and swelling joint scores after first and second
    injection in 30 μg group
  Decreased joint scores after all three injections in 100 μg group
  Decreased joint scores only after first injection in 300 μg group

Conclusions

-More than 40% of RA patients had immune response to BV17
 CDR2 peptide
-Apparent clinical benefit at all doses, but 100 μg best
-Clinical effect lasts 16–24 weeks after last peptide boost

of the BV17 and BV14 CDR2 peptides discussed above, in addition to a BV3 CDR2 peptide that had not been tested independently (Tables XII and XIII) (98). In both trials, patients received monthly injections of the peptide/IFA vaccine for 3 months, and a subsequent booster injection after an additional 3 months. In the first trial involving 99 RA patients, the 30 μg dose of each peptide (90 μg total dose) showed significant improvement at weeks 20–24 in ACR (20) scores, number of tender and swollen joints, and global assessment and pain scores. Similar but less pronounced trends were observed using the higher 100μg dose of each peptide (300 μg total dose, Table XII). Immunological responses to the combination of these peptides produced a similar rate of reactivity (40%) as was observed in earlier phase I trials using single peptides. This lack of additive effects between the peptides

might be explained by the high degree of homology between BV 3, 14, and 17 (23) and thus possible cross-reactivity among the three CDR2 peptides used. Strength and duration of the proliferation responses were modest. Circulating levels of total and activated BV3+, BV14+ and BV17+ T cells did not change, and there was no apparent correlation between response to peptide and clinical benefit. As discussed above, the delayed (>24 hr) analysis of blood samples implicit in large multi-center trials is problematic, and may have precluded accurate assessment of immunological responses.

In the second, larger multi-center trial of 340 RA patients, 10 μg and 30 μg doses of 20 mer BV3, BV14, and BV17 peptide combinations in IFA (30 μg and 90 μg total doses) were injected as above (Table XIII). In addition, the same doses of 40 mer peptide combinations were injected to evaluate if the longer peptide sequences (all of which contained the shorter peptide sequences within their structures) would be more active immunologically and clinically. In this 24 week trial, the lower dose of 20 mers produced significant clinical benefit (ACR20) versus adjuvant alone 4–8 weeks after

Table XI. Two Center Phase I Trial of Safety and Immunogenicity of BV14 CDR2 Peptide in Patients with Rheumatoid Arthritis

Study Design

-Treatment regimen

17 RA patients, BV14 CDR2 peptide

Two i.m. injections, 30, 100, or 300 μg peptide/IFA one month apart

-Monitoring over 24 weeks

T cell proliferation responses to peptide

Total and activated (CD25+) BV17+ T cells in PBMC

Clinical (joint scores)

Study Results

-Safety

TCR peptide immunization appeared to be safe and well tolerated

Only 2/24 adverse events (pain at injection site and pruritus) were peptide related

-Immunological responses

35% of injected patients had increased proliferation responses to BV14 peptide

-Clinical responses

Decreased pain and swelling joint scores at all time points in 30 and 100 μg groups

Improvement in joint scores at week 8 but not week 24 in 300 μg group

Conclusions

-BV14 CDR2 peptide is immunogenic in about 35% of RA patients tested

-Clinical efficacy best in 30 and 100 μg groups, and lasts at least 20 weeks after peptide boost

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Table XII. Multicenter Double Blind Adjuvant-Controlled Trial of TCR Peptide Vaccination in Rheumatoid Arthritis Using a Combination of BV3, BV14, and BV17 CDR2 Peptides (98)

Study Design

-Treatment regimen

99 RA patients, BV3, BV14, and BV17 CDR2 peptides

90 μg (30μg each) or 300 μg (100 μg each) peptide/IFA; IFA control

Four i.m. injections (weeks 0, 4, 8, and 20)

-Monitoring over 32 weeks

T cell proliferation responses and antibodies to each peptide

Total and activated (CD25+) BV3, BV14, and BV17+ T cells in PBMC

Clinical (joint pain and swelling scores, Health Assessment and ACR scores)

Study Results

-Safety

TCR peptide immunization appeared to be safe and well tolerated

No serious peptide-related adverse events

-Immunological responses

Sporadic proliferation responses observed in about 35% of patients

Responses tended to be highest in 300 μg dose group

No correlation between T cell response to peptide and clinical benefit

No antibody responses to peptide

Circulating levels of total and activated BV17+ T cells remained constant

-Clinical responses

Significant ACR (20) response in 90 μg group at weeks 20 and 24

Significant improvement in tender and swollen joints for 90 μg group at weeks 20 and 24

Significant improvement in global assessment and pain scores for 90 μg group at week 24

Similar but insignificant trends in 300 μg group

Conclusions

-Proliferation responses observed were sporadic, short lived and did not correlate with clinical response

-90 μg dose (30 μg of each peptide) had significant clinical effects (ACR20) versus adjuvant controls

-90 μg dose more effective clinically than 300 μg dose

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both the third and fourth injections. The higher dose of 40 mers, similar to low dose of 20 mers in molar concentration, showed clinical benefit 4 weeks after the fourth injection. Of interest, evaluation of the entire data set suggested that clinical benefit was essentially equivalent for both lengths of peptide, indicating the longer peptides did not contain additional determinants nor a more potent form of the determinant contained in the shorter peptides. Overall, the vaccines were more reactive in patients with early disease than in those with disease >10 years. Immunologically, again moderate responses with short duration were seen, with an apparent correlation with clinical benefit in patients with SI's > 10.

The clinical results to date raise important questions about possible mechanistic differences of TCR peptide reactive T cells in RA versus MS. In MS, the optimal treatment dose was between 100–300 μg peptide, and there appeared to be an association between the degree of T cell response to BV5S2 peptide and clinical benefit. In contrast, the optimal treatment dose in RA was between 30–90 μg total peptide, and there was not a clear association between the degree of T cell response to the TCR peptide combination and

clinical benefit, although beneficial trends in clinical responses were observed in the few patients who showed strong T cell responses to the peptides. One possible explanation for this apparent disparity is that cytokine profiles of the TCR responsive T cells might be peptide and dose dependent. Thus, BV5S2 CDR2 peptides might induce higher levels of IL-10 or other anti-inflammatory cytokines upon optimal stimulation, whereas BV3, BV14, and BV17 CDR2 peptides might induce less or different anti-inflammatory cytokines at higher versus lower doses. A second consideration is that later stages of arthritis, when joint inflammation

Table XIII. Multicenter Double Blind Adjuvant-Controlled Trial of TCR Peptide Vaccination in Rheumatoid Arthritis Using a Combination of BV3, BV14, and BV17 20 mer or 40 mer CDR2 Peptides (in Preparation)

Study Design

-Treatment regimen

  340 RA patients, BV3, BV14, and BV17 CDR2 peptides, 17–20 mers or 40 mers

  30 μg (10 μg each) or 90 μg (30 μg each) peptide/IFA

  Four i.m. injections (weeks 0, 4, 8, and 20)

-Monitoring over 24 weeks

  T cell proliferation responses and antibodies to each peptide

  Delayed type hypersensitivity (DTH) responses to peptides

  Clinical (joint pain and swelling scores, Health Assessment and ACR scores)

Study Results

-Safety

  TCR peptide immunization appeared to be safe and well tolerated

  3/27 serious adverse events were possibly related to study treatment with 90 μg 40 mer

-Immunological responses

  Higher proliferation responses observed in both dose groups compared to controls

  Responses were generally moderate (SI < 10) and short term

  Patients with SI's > 10 showed higher clinical responses (7/10 > ACR30)

  DTH responses to peptides sporadic

  No antibody responses to peptides

-Clinical responses

  Significant ACR20 response in 20 mer 30μg group versus control at week 16

  Clinically meaningful ACR20 improvement in 20 mer 90 μg group at week 12

  Clinically meaningful ACR20 improvement in 20 mer 30 μg group at week 24

  Clinically meaningful ACR20 improvement in 40 mer 90 μg group at week 24

  Significant response to 20 mers (30 μg and 90 μg) in patients with early disease

Conclusions

-30 μg 20 mers and 90 μg 40 mers demonstrated significant therapeutic effects

-40 mer peptides appeared to be more immunogenic than 20 mers

-Improvements in both strength and duration of immunological responses should increase clinical responses

-Subgroups of patients showing ACR50 responses and those showing <ACR10 responses show potential of approach but need for improved vaccines

-Monthly injections and enrollment of patients with disease <10 years may improve clinical outcome

involving non-T cell components is well established, may be more refractory to T cell regulation. Clearly, an assessment of the frequency and cytokine profiles of TCR reactive T cells in the synovium of vaccinated RA patients who showed excellent clinical benefit (ACR50) versus those with minimal benefit (ACR < 20) would be valuable in establishing local effects of TCR peptide vaccination.

TCR Studies in Psoriasis Vulgaris

Psoriasis is a T cell-mediated autoimmune disease of the skin in which the pathology is complex but clearly involves activated T lymphocytes. The chronological steps in lymphocyte activation leading to the development of the psoriatic lesion are thought to include initial systemic activation and induction of specific CD4+ T cells, with infiltration and local accumulation of these specific CD4+ T cells in the skin, followed by recruitment of non-specific CD4+ lymphocytes and monocytes, and finally clonal intra-epidermal expansions of CD8+ lymphocytes (99).

There are several compelling lines of evidence for T cell involvement in psoriasis, including the initiation of psoriatic lesions in immunodeficient mice after transfer of superantigen or IL-2 activated peripheral blood leukocytes from psoriasis patients (100,101). In addition, intra-epidermal CD8+ T cells isolated from plaque regions were found to be oligoclonal, expressing BV3 and BV13S1 genes in their TCRs (102,103). Finally, early stage elimination of activated T cells using IL-2 fusion toxin has therapeutic benefit (104,105).

The over-expression of limited BV genes by CD8+ T cells provided the impetus for two TCR peptide vaccination studies using CDR2 peptides from BV3 and BV13S1 sequences. These studies involving a total of 177 psoriasis patients are listed in Table I and outlined in Tables XIV and XV. In the first study, 93 psoriasis patients received 3 monthly injections of 30 μg or 100 μg of each peptide in IFA (60 μg or 200 μg total dose), or IFA alone, followed by a booster injection after another 3 months, and then were monitored for an additional 3 months (Table XIV). Similar to the MS studies, these TCR peptide vaccines induced measurable increases in T cell proliferation responses in both treatment groups, but no skin test or antibody responses were noted. As was observed in all of the above studies, the peptide injections were safe and well tolerated. Unexpectedly, patients in all groups including the control group improved clinically, due probably to initial high rates of seasonal flares and spontaneous remissions that were not treatment related.

The second psoriasis trial in 84 patients utilized 3 monthly injections of a single dose of BV3 and BV13S1 peptides (50 μg of each peptide, 100 μg total dose), and compared responses to 20 mers versus

Table XIV. Multicenter Double Blind Adjuvant-Controlled Trial of TCR Peptide Vaccination in Psoriasis Vulgaris Using a Combination of BV3 and BV13S1 CDR2 Peptides (Unpublished).

Study Design

-Treatment regimen

93 Psoriasis patients, BV3 and BV13S1 CDR2 peptides

60 μg (30 μg each) or 200 μg (100 μg each) peptide/IFA

Four i.m. injections (weeks 0, 4, 8, and 20)

-Monitoring over 32 weeks

T cell proliferation responses and antibodies to each peptide

Delayed type hypersensitivity (DTH) responses to peptides

Total and activated (CD25+) BV3+ and BV13S1+ T cells in PBMC

Clinical grading of lesions and Psoriasis Area and Severity Index (PASI)

Study Results

-Safety

TCR peptide immunization appeared to be safe and well tolerated

No differences in treatment and control groups in incidence of adverse events

-Immunological responses

Increased proliferation responses to peptides observed in 35% of peptide treatment groups

No DTH responses to peptides

No antibody responses to peptides

-Clinical responses

Patients in all groups showed improvement in lesion scores

No differences among treatment groups and controls in lesion and PASI scores

Conclusions

-Proliferation responses induced in peptide-treated groups versus controls

-Clinical improvement attributed to initial high rate of seasonal flares and spontaneous remission

-No safety issues

Table XV. Multicenter Double Blind Placebo and Adjuvant-Controlled Trial of TCR Peptide Vaccination in Psoriasis Vulgaris Using a Combination of BV3 and BV13S1 20 mer or 40 mer CDR2 peptides (Gottlieb et al. in Preparation)

Study Design

-Treatment regimen

84 Psoriasis patients, BV3 and BV13S1 CDR2 20 mer or 40 mer peptides

100 μg (50 μg each) peptide/IFA, peptide/Detox ™PC, or peptide/Saline, or vehicles alone

Three i.m. injections (weeks 0, 4, 8)

-Monitoring over 16 weeks

T cell proliferation responses and antibodies to each peptide

Delayed type hypersensitivity (DTH) responses to peptides

Clinical grading of lesions and Psoriasis Area and Severity Index (PASI)

Study Results

-Safety

Higher number of adverse events in Detox ™PC and Saline groups versus IFA group

Overall, TCR peptide immunization appeared to be safe and well tolerated

-Immunological responses

Increased proliferation responses to peptides in both peptide/adjuvant treatment groups

Immunogenicity of 40 mers/adjuvants > 20 mers/adjuvants > adjuvants or peptides/saline

DTH responses to peptides sporadic but not different among groups

No antibody responses to peptides

-Clinical responses

Clinically meaningful PASI changes in IFA group (40 mer, 28%; 20 mer, 17%; IFA, 7%)

No clinically meaningful changes in Detox ™PC or Saline groups

No significant difference in target lesion score improvement in any treatment group

Conclusions

-Maximal clinical effects (25% reduction of PASI scores) observed with 40 mers in IFA within 4 weeks of peptide booster injection

-Immunological response to peptide/IFA related to 20% PASI changes (40 mer = 20 mer > controls)

-Peptides in Detox ™PC showed higher rates of adverse reactions, were less immunogenic and yielded lower clinical benefits (PASI)

-Peptides in saline weakly immunogenic with positive clinical trend

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40 mers delivered in IFA, a Detox ™PC adjuvant, or saline over 16 weeks (Table XV). A shorter trial length and the time of year to initiate the trial were chosen to help minimize the seasonal flares and spontaneous remissions observed in the first trial. Patients injected with 20 mer or 40 mer peptides in IFA had measurable but sporadic T cell proliferation responses to peptide, with the 40 mers appearing to be somewhat more immunogenic than the 20 mers. Peptides in saline were less immunogenic. Overall, significant immunological responses to peptide in either adjuvant were related to a 20% decrease in the psoriasis area and severity index (PASI). However, because of a greater incidence of injection site reactions and adverse events, and the apparent lack of clinical efficacy noted in the PASI and lesion scores for peptides administered in Detox ™PC, it was concluded that this adjuvant would not be a good candidate for future TCR vaccination studies.

Overall, these early studies suggest a modest clinical effect of TCR peptide vaccination in psoriasis. These results are not too surprising given the current knowledge we now have on lymphocyte activation and the relatively late appearance of targeted CD8+ T cells in the psoriatic lesions. These late stage conditions make down-regulation of the pathogenic T cells difficult. An alternative strategy for use of TCR peptide vaccination in psoriasis might be to target CD4+ T cells

that appear early in the lesions at a time when PASI scores are low. However, the lack of known disease-associated tissue antigens has complicated identification of appropriate V gene targets expressed on CD4+ T cells. Identification of V genes expressed by activated T cells in the lesions is possible, but will require additional effort. Under these conditions, down-regulation of the specific early activated CD4+ and CD8+ T cells in the lesions may be possible using TCR peptide vaccines. An example of this strategy might be to utilize available agents that have broader but transient effects on PASI scores, (such as anti-LFA antibody), followed by vaccination with TCR peptides to retard formation of new lesions and delay time to flare.

Conclusions and Prospects for Future Studies

Although there were substantial variations in experimental design and outcome of the 14 clinical trials discussed above, there are a number of important conclusions that can be drawn from the combined data. Foremost is the fact that TCR peptides administered intradermally in saline or intramuscularly in IFA are safe and well tolerated. There were very few adverse events other than mild injection site reactions that were clearly related to peptide treatment, and in no case did peptide injection significantly worsen disease severity. Secondly, immunological responses, particularly proliferation of PBMC, were induced in a variable proportion (35–90%) of patients injected with peptide, with low or absent responses detected in untreated or placebo treated patients. In many cases, the measurable T cell responses were of relatively low magnitude and short duration, and it is therefore not surprising that correlations with clinical benefit occurred mainly in patients with robust T cell responses. Based on animal data and the relatively low doses needed to trigger T cell responses, it is likely that the TCR peptides are boosting pre-existing TCR responses, rather than inducing responses de novo. The major outstanding issue regarding activation of TCR reactive T cells is the regulatory mechanism. The data from the MS studies clearly implicate cytokine regulation, with secretion of IL-10 as the predominant regulatory cytokine induced by BV5S2 CDR2 peptides. Recent unpublished data from our laboratory indicate that most healthy individuals have substantial innate frequencies of IL-10 secreting cells to most CDR2 peptides from the AV and BV gene repertoire, with generally lower frequencies of IFN-γ secreting cells. Interestingly, the cytokine profiles appear to be peptide dependent, raising the possibility that a given peptide might induce higher or lower relative levels of anti-inflammatory cytokines that are needed to down-regulate autopathogenic T cells. Moreover, the inflammatory milieu in affected organs in a given disease or even in individual patients might further influence the cytokines produced by TCR-reactive T cells. In particular, the lower effective doses of TCR peptides observed in RA patient trials could be ascribed to either peptide-dependent effects or influence of elevated levels of inflammatory cytokines in joint tissue.

A related question is whether the choices of V gene targets used to date are appropriate or sufficient for a given autoimmune disease. This question has been addressed partially in each of the three diseases studied here. Generally the chosen V gene targets were derived from relatively small samplings of antigen-specific or previously activated T cells from affected tissues or peripheral blood from small numbers of patients. There is clearly some degree of patient to patient variation in expression of disease-related V genes, creating a challenge for designing TCR vaccines. Our strategy to address V gene heterogeneity partially relies on a bystander mechanism. Our goal has been to identify several disease associated V genes, and then to inject the appropriate combinations of BV peptides in the hope of having at least some represented V gene in any given patient. Given that the possible "bystander suppression" of non-targeted inflammatory cells in the local vicinity of activated regulatory T cells is implicit in the mechanism of secreted anti-inflammatory cytokines, this strategy of peptide "cocktails" could provide sufficient regulation to provide clinical benefit. However, the degree of bystander suppression possible is again likely to be peptide dependent, and this important concept still needs to be formally demonstrated in vivo.

The final important aspect of these studies is the apparent clinical efficacy of TCR peptide injections. Significant therapeutic benefits occurred in a cohort of peptide vaccinated patients versus controls in each of the three autoimmune diseases studied, and in MS these changes could be related to immunological responsiveness to the peptides. Additionally, beneficial clinical trends were observed in strong immunological responders to TCR peptides in patients with RA and psoriasis. This is a key observation, because it provides the rationale to identify or design more immunogenic peptides that can induce stronger and longer-lasting T cell responses in a higher percentage of patients. With continuing peptide development that can be evaluated in phase I studies of immunogenicity, future clinical studies that involve stronger T cell responses in a higher percentage of patients would be expected to show more

impressive clinical benefit. Conceivably, with appropriate screening techniques and a battery of reactive TCR peptides, it may be possible to induce maximal clinical responses in most patients. Under these most favorable conditions, the ultimate clinical potential of TCR peptide therapy can be fairly evaluated.

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