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*J Immunol* published online 11 May 2015
http://www.jimmunol.org/content/early/2015/05/09/jimmunol.1402457

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Immune Tolerance Induction with Multiepitope Peptide Derived from Citrullinated Autoantigens Attenuates Arthritis Manifestations in Adjuvant Arthritis Rats

Smadar Gertel,* † Guy Serre,‡ Yehuda Shoenfeld,* † and Howard Amital* † §

Citrullinated peptides are major targets of disease-specific autoantibodies in rheumatoid arthritis. Currently, citrullinated peptides are used as biomarkers for diagnosing rheumatoid arthritis by measuring anti-citrullinated protein Ab (ACPA) titers in patients’ sera. The accumulation of citrullinated proteins at synovial inflammation sites suggests that they are possible targets for tolerance induction. The objective of the present study was to determine whether citrullinated peptides could induce tolerance in an experimental arthritis model in rats. In view of the multiplicity of target citrullinated autoantigens described for ACPA, we generated a multiepitope citrullinated peptide (Cit-ME), derived from major prevalent citrullinated autoantigens (citrullinated filaggrin, fibrinogen, vimentin, and collagen type II), and studied its effects on arthritic rats. Adjuvant-induced arthritis was induced in Lewis rats. Beginning at day 7 after disease induction, the rats received eight s.c. injections of Cit-ME on alternate days. Differences in clinical status and modulation of T cell populations were analyzed. In adjuvant-induced arthritis rats treated with Cit-ME, disease severity was significantly reduced compared with that of untreated rats. Moreover, amelioration of disease manifestations was related to an increased regulatory T cell subset and an elevated apoptosis rate of T cells associated with reduced Th17 cells. Thus, the use of citrullinated peptides–based immunotherapy may be a promising approach for tolerance induction in experimental arthritis and perhaps even in susceptible individuals that are ACPA-seropositive in human arthritis. The Journal of Immunology, 2015, 194: 000–000.

Rheumatoid arthritis (RA) is an autoimmune disease characterized primarily by progressive synovial inflammation, resulting in irreversible joint destruction (1). The etiology of RA is not fully understood, yet it is speculated that a complex interplay between genetic and environmental factors is involved in the pathogenesis of the disease (2). Many of the RA-specific autoantibodies are generated against citrullinated Ags and termed anti-citrullinated protein Abs (ACPA) (3). Furthermore, their presence is one of the significant items of the new American College of Rheumatology/European League Against Rheumatism RA classification criteria (4). ACPA can be detected in almost 80% of RA patients with a diagnosis specificity reaching 98% (5), and they were reported to be more specific than rheumatoid factor for diagnosing RA (6). Additionally, ACPA can be detected years before the onset of symptoms (7) and are associated with a more erosive disease course and poor remission rate (8). The major targets for ACPA are citrullinated peptides, peptides that underwent posttranslational conversion of arginine- to citrulline-based residues. Presently, citrullinated peptides serve merely as a biomarker for diagnosis of the arthritic disease.

In RA, many proteins, such as filaggrin, fibrinogen, vimentin, and collagen type II, are subjected to citrullination (9). Furthermore, inflamed joints of RA patients contain three times more citrullinated proteins within erosive tissue compared with nonerosive tissue (10). Autoreactivity toward citrullinated peptides may contribute to the development of RA in susceptible individuals. The hypothesis that ACPA response is T cell mediated is supported by several facts. The enzyme that catalyzes citrullination, peptidylarginine deimination, is not expressed in the thymus (11), and therefore T cells reactive to citrullinated Ags are not likely to be eliminated, leaving an option of future possible immune reaction against citrullinated Ags. Additionally, ACPA formation correlates with specific HLA-DRB1, -DRB4, and -DRB10 alleles (12, 13). These alleles encode for a specific peptide-binding pocket on the APCs, the so-called shared epitope (14). Citrullination could increase self-antigen immunogenicity through increased binding affinity to shared epitope–containing HLA-DRB molecule. The latter results in loss of tolerance to citrullinated Ags with a consequent immune response to citrulline-specific autoreactive T cells, activation of immune cells, and production of inflammatory cytokines that eventually lead to synovial inflammation (12, 15–17).

Tolerance to citrullinated peptides has been shown with DBA mice. These mice are susceptible to arthritis and were found to produce genuine ACPA autoantibodies upon disease induction. Prophylactic administration of a citrullinated filaggrin peptide protected these animals from full development of arthritis by prevention of epitope spreading of ACPA reactivity (18).

Ag-induced peripheral tolerance is a promising approach for treatment of autoimmune diseases. Among the numerous approaches that have been proposed for immunotherapy of RA, the...
immune-specific approach, which specifically neutralizes pathogenic arthritic-reactive T cells and restores abnormal immune responses while leaving other immune cells intact, is the ultimate goal in disease-specific treatment.

One way to induce immune tolerance is by manipulating the interactions of APCs with lymphocytes. The immune system is normally tolerant to self-antigens; failure to do so may result in autoimmunity. The recognition of autoantigens displayed on MHC class II—expressing T cells induces partial signals in Ag-specific T cell clones, including clonal deletion and clonal anergy.

An Ag-specific approach for suppression of arthritis by derivatives of citrullinated peptides is based on the use of synthetic peptides. The selection of peptides for immunomodulation is a crucial step in this approach. Induction of Ag-specific tolerance based on the persistence of an Ag can be achieved after administration of repeated low doses (19, 20). Such interventions have aimed to immunomodulate essential T cell populations by either depleting pathologic CD4+ T cells (mainly Th1 and Th17) or by suppressing their function via the expansion of CD4+Foxp3+ regulatory T cells (Treg) populations and by downregulation of proinflammatory cytokine expression.

Experimental adjuvant-induced arthritis (AAIA) in rats is a reliable model of arthritis and suitable for investigating the mechanisms underlying the pathophysiology of RA. In order to augment the possible tolerogenic activity of citrullinated peptides in arthritic rats, we generated a multiepitope citrullinated peptide composed of sequences from the major citrullinated autoantigens, termed Cit-ME. We analyzed the effects of this peptide on disease progression and T cell responses by injecting it into rats with AIA and assessed its capacity to attenuate arthritis severity compared with peptides derived from a single prevalent citrullinated autoantigen (citrullinated β-fibrinogen).

We found that both citrullinated peptides had a therapeutic effect on arthritic rats. However, rats treated with the multiepitope peptides induced an improved profile of specific immune suppression with concomitant amelioration in their arthritis manifestations.

Materials and Methods

Serum samples

Patients with arthritis were diagnosed on the basis of the revised American College of Rheumatology/European League Against Rheumatism classification criteria. Blood samples were taken with ethical permission and after the patients had signed a written consent.

Detection of ACPA

Sera of patients were tested for ACPA using the commercial ELISA Quanta Lite CCP3 IgG kit (Inova Diagnostics, San Diego, CA), according to the manufacturer’s instructions. Sera with results <25 U/ml are defined as negative, and sera with results ≥25 U/ml are defined as positive.

Cit-ME inhibition assay by ELISA

To detect whether ACPA-specific Abs are recognized by the Cit-ME peptide, 96-well plates were coated with the following citrullinated peptides (10 μg/ml in PBS): citrullinated (cit–)collagen type II (359–369), AChLGTCCitPG-DAK; cit-flaggin (306–326), HQCHQESTCitGRSRGRCGRSGS; cit–β-fibrinogen (60–74), CitPAPPSISGGGYCitACG; and cit–vimentin (65–77), SAVRACitSSVPGRVK.

The plates were blocked with 5% BSA for 1 h at 37°C. Serum samples were added at a dilution of 1:100 in PBS 1% BSA for 4 h at room temperature. Following further washing, peroxidase-conjugated goat anti-human IgG was added at a 1:5000 dilution and incubated for 2 h at room temperature. For visualization, the chromogenic substrate 3,3′-diaminobenzidine was added. The staining reaction was stopped by addition of 1 N sulphuric acid, and absorbance at 450 and 620 nm was measured using a Bio-Tek PowerWave HT 340 plate reader (Bio-Tek, Winooski, VT). The specificity of Cit-ME binding to each specific ACPA was confirmed by inhibition assay. Serum samples from ACPA+ RA patients were selected by high positivity for ACPA in both commercial CCP3 ELISA test and positivity for each of the citrullinated peptides (i.e., Cit-ME, cit-flaggin, cit–β-fibrinogen, cit-collagen, and cit-vimentin). The selected sera were diluted in PBS to obtain a reactivity of ~50% binding OD under standard assay conditions with the Cit-ME peptide. Increasing concentrations of Cit-ME peptide were added to the diluted sera and incubated for 2 h at room temperature. Specimens were then assayed in ELISA plates coated with each of the single citrullinated peptides of which the Cit-ME peptide is composed.

Animals

Female Lewis rats, 6–7 wk of age and weighing 110–120 g, were purchased from Harlan Laboratories (Jerusalem, Israel) and housed under specific pathogen-free conditions at the animal facility of the academic Sheba Medical Center. All experiments were performed according to the institutional guidelines for animal care.

Induction of AIA and peptide treatment regimen

Arthritis was induced by a single 0.1-ml intradermal injection at the tail base of a suspension containing 10 mg/ml heat-inactivated Mycobacterium tuberculosis strain H37Ra (Difco Laboratories, Detroit, MI) emulsified in CFA (Difco Laboratories). Cit-ME (peptide sequence scheme is demonstrated in Fig. 1A), matched non-citrullinated peptide containing arginine or lysine instead of citrulline (Non-Cit-ME), citrullinated β-fibrinogen peptide (β-Fib-Cit) (21), and the matched non-citrullinated β-fibrinogen peptide containing arginine instead of citrulline (β-Fib-NC) were used. The multiepitope synthetic peptides were obtained from GL Biochem (Shanghai, China). The β-fibrinogen peptides were obtained from NeomPS (Strasbourg, France). Immediately after disease induction, animals were randomly assigned to one of the experimental treatment groups, and each group was injected with one of the above-mentioned peptides. For therapeutic treatment, doses, from day 7 on, rats with ongoing arthritis were injected s.c. with the experimental peptides. Eight injections (300 μg/injection) were given on alternate days. A group of untreated arthritic rats served as an AIA control group (n = 5–8 rats/group).

Assessment of arthritis

The severity of arthritis was assessed by measurement of swelling of the rat paws. The progression of arthritis was measured by paw diameter changes in millimeters with a digital microlcaliper measuring the thickness of the fore- and hindlimbs, respectively. Change in paw volume was presented as the mean ± SEM for each group.

Histology

The hindpaws of the experimental rats were removed and fixed in 4% paraformaldehyde in PBS and then decalcified and embedded in paraffin. For the histological analysis, sections (5 μm thick) were stained with H&E. Inflammation was quantified on H&E-stained sections using a semiquantitative score (scale of 0–3): 0, normal; 1, mild inflammation; 2, moderate inflammation; 3, marked inflammation. All sections were evaluated histologically by two independent observers.

Treg characterization

FRESHLY isolated splenocytes (2 × 106) were analyzed by using anti-CD4 and anti-CD25 mAbs (eBioscience, San Diego, CA). To evaluate intracellular Foxp3 expression, lymphocytes were stained with CD4 and anti-CD25 mAbs, then fixed and permeabilized with Foxp3 detection kit buffers. After staining with anti–Foxp3-PE (eBioscience), the lymphocytes were analyzed by flow cytometry. Data acquisition was performed with FlowJo software.

Th17 cell characterization

Splenocytes were cultured with PMA (5 ng/ml) and ionomycin (1 μM) (Sigma-Aldrich, Seelze, Germany). Cytokine secretion was inhibited by brefeldin A (eBioscience). Surface staining was performed with CD4 followed by fixation and permeabilization kit (eBioscience) and stained with anti-CD17 Ab, and cells were analyzed by flow cytometry.

Assessment of apoptosis

Apoptosis was measured by detecting phosphatidylserine externalization in the cell membrane using the annexin V/propidium iodide assay (eBioscience). The assay procedure was performed according to the manufacturer’s instructions. Briefly, splenocytes were stained with anti-CD4. Following incubation, annexin V labeling solution was added and allowed to incubate for 15 min in the dark at room temperature, propidium iodide was added before the last washing, and then cells were analyzed by flow cytometry.
Real-time RT-PCR

Total RNA was isolated from spleen-derived lymphocytes of the experimental rats. The RNA was reverse transcribed to prepare cDNA using Moloney murine leukemia virus reverse transcriptase (high-capacity cDNA reverse transcription kit, Invitrogen, Carsbad, CA). The resulting cDNA was subjected to real-time RT-PCR performed on a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. Primer sequences (forward and reverse, respectively) were: rat IL-17, 5′-TGGCGGTTCCTTCCTCAGTC-3′, 5′-CGGTTGACTCTTCTATCC-3′; FAS, 5′-TGCTGCCTGGCTCTTG-3′; 5′-CGAACGCTCTCTCTCAACCTC-3′; IL-6, 5′-CGCTTCTACGAAACAGCTTGAGA-3′, 5′-TGGCAACAACATCATCGCCGAAG-3′; and β-actin, 5′-ACGTTGAAAGATGACCCAGAT-3′, 5′-AAACCCTCAAGTGGGCAAG-3′. The levels of β-actin were used to normalize the gene expression levels of the gene of interest.

Statistical analysis

Differences in mean values between experimental groups were assessed with the Student t test. A p value < 0.05 was considered significant.

Results

The generation of multiepitope citrullinated peptide and its reactivity with specific ACPA

In view of the diversity of citrullinated target autoantigens in RA, we generated a multiepitope citrullinated peptide derived from the sequence of the best known autoantigens that bind ACPA (e.g., epitopes derived from citrullinated filaggrin, fibrinogen, vimentin, and collagen type II). The peptide was designated Cit-ME. Its composition is based on sequence juxtaposition to the citrullinated regions of the peptides that are considered to be highly reactive and commonly used in ACPA testing (Fig. 1A). The cross-reactivity of Cit-ME peptide binding to different ACPA (cit-filaggrin, cit-β-fibrinogen, cit-collagen, and cit-vimentin Abs) was confirmed by peptide inhibition assay in ELISA (Fig. 1B). Ten to 200 μg Cit-ME peptide inhibited the binding of the specific ACPA (cit-filaggrin, cit-β-fibrinogen, cit-collagen) and to a lesser extent of cit-vimentin. Only the Cit-ME could inhibit the specific ACPA whereas the Non-Cit-ME peptide could not (data not shown).

Clinical effects of citrullinated peptides on arthritis severity in rats

The experimental citrullinated peptides were tested for their tolerogenic ability following administration to AIA rats in vivo. The effects of Cit-ME on arthritis manifestations were compared with those of a single citrullinated peptide derived from highly prevalent citrullinated autoantigen sequence, namely β-Fib-Cit (21), and to the matched non-citrullinated form β-Fib-NC. Arthritis began to appear in the untreated rats from day 10 and was sustained for 28 d. Rats treated with β-Fib-NC exhibited a severe form of arthritis compared with the untreated rats (reflected by mean paw diameter). Compared to untreated rats, reduced arthritis severity scores were observed in rats that were treated with Cit-ME or β-Fib-Cit. At days 14 and 21, only rats that were treated with the Cit-ME peptide had significantly lower arthritis severity (3.43 ± 0.15 and 3.33 ± 0.13 mm) as compared with the untreated rats (3.83 ± 0.1 mm [p < 0.02] and 4.2 ± 0.19 mm [p < 0.01], respectively) (Fig. 2A). Cit-ME–treated rats continued to exhibit a significantly reduced mean paw diameter (3.09 ± 0.18 mm) as compared with the other experimental groups (untreated, 4.07 ± 0.2 mm; β-Fib-NC, 4.2 ± 0.32 mm; and β-Fib-Cit, 3.66 ± 0.17 mm; p < 0.01) at the end of the experiment (day 28). Representative images of experimental rat hindpaws are shown in Fig. 2B.

The histological analyses of joints inflammation of the experimental rats groups reflected the clinical assessments (data not shown). The efficacy of Cit-ME in attenuation of AIA was then assessed and compared with administration of the matched non-citrullinated peptide. Rats were treated with Cit-ME, Non-Cit-ME, or were untreated. As shown in Fig. 3A, in untreated rats, the signs associated with the development of arthritis began gradually and were evident on day 14 after disease induction. Later, the arthritis progressed and reached the highest severity score on day 21 and remained constant until day 28. Treatment with Cit-ME peptide reduced significantly the mean paw diameter (3.66 ± 0.13 mm) compared with the untreated rats (4.61 ± 0.17 mm, p < 0.002) at day 14. The Cit-ME peptide–treated group demonstrated significantly less swelling on days 21 and 28 after disease induction (3.68 ± 0.13 and 3.74 ± 0.13 mm) as compared with the untreated (4.62 ± 0.2 mm, p < 0.001; 4.59 ± 0.18 mm, p < 0.002) and Non-Cit-ME–treated rats (4.07 ± 0.13 and 4.2 ± 0.16 mm, p < 0.03), respectively. Treatment with the Non-Cit-ME peptide showed a trend toward reduction of arthritis, but statistically significant differences were not observed when compared with untreated rats (Fig. 3A). Significant visible differences were evident in rats treated with the Cit-ME peptide on day 27 after disease induction (Fig. 3B).

A major reduction of inflammation indices was demonstrated in joint sections of Cit-ME peptide–treated compared with joint sections of untreated and Non-Cit-ME peptide–treated rats. Fig. 3C shows representative joint sections from rats in the different groups. Untreated arthritic rats had a higher histological inflammatory score (1.75 ± 0.41), whereas rats treated with the Cit-ME peptide had a significantly lower score (0.54 ± 0.2) (Fig. 3D). As shown in Fig. 3D, treatment with the Non-Cit-ME peptide did not reduce significantly the inflammatory index (1.29 ± 0.6) as compared with the untreated rats.

Upregulation of Tregs in Cit-ME–treated rats

Treatment with the Cit-ME peptide was investigated for its potential immunomodulatory effects on Tregs in arthritic rats. The proportion of CD4+CD25+Foxp3+ Treg population in spleens of the experimental rats was determined by flow cytometry. Fig. 4A shows representative plots for CD4+ gated cells that were stained for CD25 and Foxp3. A significant increase (p < 0.01) in the percentage of the CD4+CD25+Foxp3+ Treg subset was observed in the spleens of Cit-ME–treated rats (8.13 ± 0.17) compared with
Non-Cit-ME peptide–treated and untreated rats (6.97 ± 0.22 and 7.17 ± 0.31, respectively) (Fig. 4B). Analysis of the percentage of splenic Treg population in Cit-ME–treated, β-Fib-Cit peptide–treated, and untreated rats (n = 5–6 rats/group) indicated that both citrullinated peptides upregulated the Treg population as compared with the untreated rats (data not shown).

Reduced IL-17+CD4+ T cell population in Cit-ME–treated rats

To evaluate the effect of Cit-ME on T cells, we assessed the distribution of the pathogenic IL-17+CD4+ T cells in the experimental rats. The percentage of IL-17+CD4+ T cells was significantly decreased (p < 0.03) in Cit-ME–treated (0.68 ± 0.1) compared with untreated rats (1.14 ± 0.2) (Fig. 5A, 5B). Similarly, as shown in Fig 5C, a significant decrease in the percentage of IL-17+CD4+ T cells was determined in Cit-ME–treated versus that detected in the untreated rats. The frequency of IL-17+CD4+ T cells was slightly reduced in the β-Fib-Cit–treated relative to untreated rats, but this was statistically insignificant.

To further confirm the difference in the frequency of IL-17+CD4+ T cells, we performed quantitative RT-PCR to validate the IL-17 and IL-6 expression in the spleen of the experimental rats. Treatment with Cit-ME resulted in significant reduction in the mean relative expression level of the IL-17 and IL-6 mRNA in comparison with that observed in the untreated rats (Fig. 5D).

Increased T cell apoptosis in Cit-ME–treated rats

The effects of the various peptides on T cell apoptosis in the arthritic rats was assessed and, as shown in Fig. 6A–C, a significantly higher mean apoptosis rate was found in splenic CD4+ T cells of Cit-ME–treated rats (19.5 ± 1.8%) compared with the cells of Non-Cit-ME–treated and untreated rats (13.8 ± 1.3 and 13 ± 1%, respectively). We determined whether the observed apoptosis is associated with the apoptosis-inducing receptor pathway and measured the mRNA expression of the FAS gene in rat splenocytes from the above groups. As shown in Fig. 6D, the mean relative expression level of FAS mRNA was significantly higher
Discussion
The present study demonstrated that administration of a multi-epitope peptide expressing sequences derived from four arthritic-related citrullinated autoantigens induced immune tolerance, was able to modulate T cell responses, and ameliorated the clinical status of arthritic diseased rats.

The administered citrullinated peptides are presumably processed and presented by APCs to induce mechanisms that control citrulline-specific autoreactive T cells. Induction of peripheral tolerance using synthetic peptides delineated from self-autoantigen sequences were previously shown to suppress disease manifestations in arthritic models (22, 23). The tolerance mechanisms include clonal deletion of autoreactive T and B cells (24), clonal anergy (25), and induction of T cells with a regulatory phenotype (26).

In multiple sclerosis, the inflammatory immune response is directed against myelin Ags of the CNS. Experimental autoimmune encephalomyelitis (EAE) is a mouse model of multiple sclerosis used to study disease pathophysiology and treatment. Treatment with a truncated form of myelin peptide was able to induce neuroantigen-specific tolerance and reverse EAE. The short peptide, which is too small to stimulate an antigenic response to pathogenic regions of myelin basic protein, is however effective as a tolerogen and is able to anergize autoreactive T cells (27). Also, in systemic lupus erythematosus, a tolerogenic peptide designated hCDR1 (28), which is based on the sequence of the H chain CDR...
of a human monoclonal anti-DNA Ab (29), ameliorated significantly systemic lupus erythematosus manifestation in lupus mice (30, 31). The hCDR1 that was administrated in small doses (25 or 50 μg) s.c. once a week was found to improve the clinical manifestations of lupus mice through the induction of Tregs that suppress activation of the autoreactive cells (32).

In view of the diverse citrullinated autoantigen targets and ACPA repertoire found in RA, the autoimmune response is widely extended to different citrullinated epitope targets in each individual. Moreover, the primary target Ag may differ among arthritic patients. An autoimmune reaction against a certain citrullinated protein may expand beyond the original epitope to involve other citrullinated Ags as well as the disease progressions. Thus, by the time the disease is diagnosed, a complex of pathogenic autoimmune networks is already established. Because the autoimmune responses in established RA are directed against a complex of citrullinated autoantigens, treatment with a multiepitope peptide may increase the odds of neutralizing pathogenic autoreactive T cells that differ by their affinities.

Likewise, in EAE, administration of a multiple-epitope protein containing a sequence of five known major encephalitogenic target autoantigens (33), as well as the utilization of a peptide mixture of four distinct encephalitogenic epitopes (34), was found to inhibit experimental autoimmune encephalomyelitis. Implementation of this principle was shown to be more effective than treatment with a single Ag, probably by neutralizing concomitantly T cells reactive against different encephalitogenic targets, promoting the chances of achieving wider peripheral tolerance.

The citrullinated peptide Cit-ME significantly attenuated disease severity whereas treatment with the β-Fib-NC exacerbated the arthritis severity as compared with the untreated rat group (Fig. 2). Administration of the Non-Cit-ME peptide comprising the same amino acid sequence also resulted in amelioration in disease severity, although not to the same degree (Fig. 3) as did the Cit-ME peptide. Alternatively, administration of a peptide containing an unmodified arginine residue might result in in vivo citrullination by the peptidylarginine deiminase enzyme and enhance the immune response against the native peptide as demonstrated in our study with the β-Fib-NC (Fig. 2).

Abs to citrullinated proteins were detected in induced rat and mouse models of arthritides during the course of disease development (35, 36). We could not detect a decrease in the ACPA levels in the experimental rats using the commercial CCP3 ELISA. It might be that the rapid emergence of AIA manifestations as well as the rat relative short course of disease and longevity as designed in this study did not provide sufficient time to detect such an association.

This study shows that the Treg subpopulation is upregulated in Cit-ME–treated rats. The clinical improvement following treatment with the multiepitope peptide correlated with the observed upregulation of the polyclonal Tregs.

The CD4+CD25+Foxp3+ Tregs are capable of suppressing autoreactive T cells in various types of autoimmunity. It would be of interest to test whether Treg cells from Cit-ME–treated rats would possess higher suppression capacity. Tregs are considered to be useful for suppression of autoimmune diseases, although evidence suggest this will only be effective when the Treg population is specific to the relevant Ag (37). It is also important to characterize whether the citrulline-specific Treg subset was enriched in the Cit-ME–treated rats.

An increased Treg/Th17 ratio was demonstrated following treatment of AIA rats with Cit-ME. The CD4+ Th cell subset of Th17 cells and its effector cytokine IL-17 were shown to promote inflammation, autoimmunity, and RA (38, 39). Several studies have examined the roles of Th17 cells, a distinct lineage of CD4+ T effector T cells, in various arthritis models (40–42). Collagen-induced arthritis was shown to be partially suppressed in IL-17–deficient mice (41). Treatment with Cit-ME showed a trend toward a reduced frequency of Th17 cells, as well as reduced mRNA levels of IL-17 and IL-6 (a cytokine that plays an essential role regulating the differentiation of Th17 pathway) in the spleens of the treated rats. Further studies are required to determine the frequency of Th17 cells in the inflamed joints and circulation following treatment with citrullinated peptides. The redistribution of inflammatory cells could be at least one of the mechanisms responsible for ameliorating disease manifestations.

Increased apoptosis of splenic T cells was also observed following treatment with Cit-ME. Our results showed that FAS mRNA expression increased significantly in the lymphocytes of tolerized rats. The binding of FAS with FAS ligand could trigger a sequence of intracellular events that initiate apoptosis of pathogenic autoreactive T cells.

Based on the present study, we suggest that the administration of citrullinated peptides and in particular a multiepitope citrullinated peptide may be considered as a way to induce Ag-specific tolerance in AIA rats. In view of these data, their potential effects in RA patients should be considered.

Disclosures

The authors have no financial conflicts of interest.

References


