T Cell Vaccination as a Tool in the Treatment of Collagen Induced Arthritis in Albino Rats

Shahnaz Rafiei[1], Forouzan Karimi[1,2*], Fatemeh Roohollah[3], Ali Rafinejad[1]

[1]Department of Immunology, School of Medicine, Tehran Medical Sciences University, Tehran, Iran, [2]National Research Center for Medical Sciences of I.R. Iran, Tehran, Iran, [3]Azad University Center for Sciences and Researches, Tehran, Iran.

ABSTRACT

Background: The effectiveness of T cell vaccination has been demonstrated in a variety of animal models of both induced and spontaneous autoimmune diseases. **Objective:** The purpose of this study was to test the T cell vaccination protocol to treat and prevent collagen induced arthritis (CIA) in a rheumatoid arthritis model. **Methods:** CIA was induced by an intradermal injection of an artheritogen substance at the right paw of each female Albino rat under ether anesthesia. T cells were achieved from spleens of syngeneic rats that developed full clinical features of CIA. Rats suffering from CIA were divided in case groups (4 rats/group) based on the degrees of their disease and were injected intraperitoneally once with a suspension of T cells to investigate the effects of autoreactive T cells on CIA. To investigate the preventive effects of autoreactive T cells on CIA, 12 normal rats were injected intraperitoneally once either with a suspension of T cells or PBS, respectively. The results were evaluated by clinical observation, histopathological and radiographic findings. **Results:** Intraperitoneal inoculation of T cells to rats suffering from CIA, suppressed the development of CIA in case rats in stage 2 of the disease but not the other case rats. Rats who received T cells as prevention, showed the mild signs of disease. Injection of artheritogen substance to the case rats didn't result in development of CIA but the control rats, showed signs of CIA. **Conclusion:** The results of this pilot study demonstrate that CIA presentations and signs can be subsided or suppressed by autoreactive T cells. The vaccination is most effective before onset of the disease and in early phases of CIA. Modifying and improving the protocol using more cases is recommended.

Key words: Arthritis, Collagen Induced Arthritis, Rats, T Cell Vaccination

*Corresponding author: Dr. Forouzan Karimi, National Research Center for Medical Sciences of I.R. Iran, No. 26, St. No. 1, Kooh-e Noor St., Motahari St., Tehran, Iran. Tel: +98(21)8501403-6 (extension: 217), e-mail: fkarimi_2001@yahoo.com

INTRODUCTION

Studies suggest that experimental autoimmune diseases could effectively be prevented and treated by application of autoreactive T cells. The effectiveness of T-cell vaccination has been demonstrated in a variety of animal models of both induced and spontaneous autoimmune diseases. T cell vaccination can be highly specific and is likely to be non-toxic, because it exploits the immune system's own ability to control its autoimmune aberration (1-4). Organ-specific autoimmune diseases are caused by autoreactive T cells that attack normal tissues of the individual (2,3). Although activated, autoreactive T cells are potentially pathogenic, however, experimental autoimmune diseases can effectively be prevented and treated by application of autoreactive T cells in a normal or an attenuated form (1,4-6). Collagen Induced Arthritis (CIA), a chronic inflammatory disease of the joints, is induced in rats by immunization with Complete Freund's Adjuvant and Type II Collagen (7,8). It has been demonstrated that clones of T lymphocytes reactive to Mycobacterium and Collagen, can not only induce but also prevent CIA (3,9,10). In the 1980s T cell vaccination protocols, designed to induce an antiidiotypic response, were found to prevent and reverse a variety of autoimmune diseases in rodents (11). In these protocols host vaccination was achieved with attenuated syngeneic T cells pre-sensitized to the target autoantigen. The idiotype of the sensitized T cells, when injected as a vaccine, theoretically would induce an antiidiotypic T cell response in the host, which, if vigorous enough, would suppress the reactivity or destroy the T cells directed against the target antigen (12).
The purpose of this study was to test in a rheumatoid arthritis model the T cell vaccination protocol to treat and prevent collagen induced arthritis (CIA) in rats. In this study, the effects of T cell vaccination on improvement the CIA was evaluated by clinical observation (reducing the symptoms), histopathological and radiographic findings.

MATERIALS AND METHODS

Animals. Inbred Albino rats were obtained from Pasteur Institute, Tehran, Iran. Rats were housed at the Azad University Center for Sciences and Researches Animal Care facilities, fed a sterile commercial diet and water ad libitum, and maintained in optimum conditions (13). At the age of 3-4 weeks, females and males were separated and only females (6-10 weeks, body weight: 250-300 g) were used in the experiments.
Antigens and adjuvant. Complete Freund's adjuvant (CFA) was purchased from Pasteur Institute, Tehran, Iran. Bovine Type II Collagen was purchased from Sigma, USA.
Induction of Collagen Induced Arthritis (CIA). Collagen Induced Arthritis (CIA) was induced by an intradermal injection of 0.1 ml of an emulsion containing CFA and 50µg bovine collagen type II at the right paw of each rat under ether anesthesia. The injections were made with the help of a fine needle (No. 22). Normal control rats were injected with 0.05 ml phosphate-buffered saline (PBS) in the right paw

(14,15).

Clinical Evaluation of CIA. The development of arthritis was assessed by the Wood et al. (16) classification. According to this classification, the following degrees were attributed to each joint:

0 = Normal
1 = Redness only
2 = Redness plus mild swelling
3 = Redness plus severe swelling
4 = Joint deforming

Rats were observed daily for the presence of clinical signs of CIA and all clinical evaluations were performed in a blinded fashion. Clinical observations were then confirmed by histological analysis and radiological findings.

The rats were divided in 5 groups as follows:

Group 1: normal and naïve rats, as control;
Group 2: rats at grade one, symptoms appearing (redness only);
Group 3: rats at grade two, clinical manifestations (redness + mild swelling);
Group 4: rats at grade three, (redness + sever swelling);
Group 5: rats at grade four, (joint deformity).

Isolation of lymphocytes. T cells were achieved from spleen of syngeneic rats that developed full clinical features of CIA as follows:

3 weeks after immunization, spleens of 10 rats affected by CIA were separated and a single suspension was prepared. Lymphocytes were separated from the suspension on a Ficoll gradient. Then, using nylon wool column, T cells were separated from B cells (17,18). Using rosette test, the presence of T cells were checked (18). T cells viability was checked by Trypan Blue staining, (19). Then the viable T cells were washed and were suspended in PBS.

After cultivating T cells in RPMI-1640 medium (sigma) containing 100 U/ml penicillin, 100 μg/ml streptomycin, $5x10^{-5}$ m 2-mercaptoethanol, 1% non-essential amino acid, and 10% heat-inactivated fetal calf serum (FCS)(sigma) for 48 hours, autoreactive T cells were activated in the presence of syngeneic peritoneal cells as antigen-presenting cells (APC) (20-22) by 10 μg/ml collagen type II (incubator 37ºC, wet atmosphere and 5% CO2) (21,23).

T Cell Vaccination.

a. Treatment

Investigating the role of autoreactive T cells in the suppression of CIA, 32 rats suffering from CIA were used as follow:

Rats at grade 1 of CIA: 4 rats as case; 4 rats as control;
Rats at grade 2 of CIA: 4 rats as case; 4 rats as control;
Rats at grade 3 of CIA: 4 rats as case; 4 rats as control;
Rats at grade 4 of CIA: 4 rats as case; 4 rats as control (21,24).

Rats in case groups (4 rats/group), were injected intraperitoneally once with a suspension of $1x10^5$ T cells in PBS/rat. The rats in control groups (4 rats/group) were injected intraperitoneally once with PBS. In order to induction of CIA, four weeks after the inoculation of autoreactive T cells, all of the rats in case and control group, were immunized with an emulsion of IFA and collagen type II. Rats were observed daily for any change and presence or absence of clinical signs of CIA. All clinical

evaluations were performed in a blinded fashion. Clinical observations were then confirmed by histological analysis.

b. Prevention

To investigate the effects of autoreactive T cells in the prevention of CIA, 12 normal rats were used (six normal rats as case, and six normal rats as control). Rats in case group were injected intraperitoneally once with a suspension of $1x10^5$ T cells/rat in PBS. Rats in control group, were injected intraperitoneally once with PBS.

With daily observations, the reaction of rats against the injections was controlled. Three weeks after inoculation of autoreactive T cells or PBS to normal rats, all the rats in case and control group were injected with artheritogen substance (an emulsion of IFA and collagen type II). Rats were observed daily for any change in their condition and presence or absence of clinical signs of CIA. All clinical evaluations were performed in a blinded fashion. Clinical observations were then confirmed by histological analysis.

Histopathology. To study the effects of autoreactive T cells on CIA and to confirm the suppression of CIA by autoreactive T cells as observed in clinical evaluation, 16 to 20 days after immunization, rats were killed and the tissue specimens were fixed in 10% formalin, decalcified in formic acid, and stained with haematoxylin and eosin (13,25,26).

RESULTS

Induction of CIA in albino rats. All of the female albino rats that were injected with the artheritogen substance (an emulsion of CII and IFA) showed the signs of CIA (Fig. 1 and 2).

Treatment of CIA using autoreactive T cells. Intraperitoneal inoculation of as low as $1x10^5$ autoreactive T cells to rats suffering from CIA (by one injection) suppressed the development of CIA in case rats in stage 2 of the disease (n = 4) but not the other case rats (Fig. 3 and 4). Clinical observations were then confirmed by histological analysis.

Prevention of CIA by using autoreactive T cells. All of the rats who received intraperitoneal inoculation of as low as $1x10^5$ autoreactive T cells, showed the mild signs of CIA (n = 6), but the other rats remained healthy. Injection of artheritogen substance to the case rats (that received autoreactive T cells before) did not result in development of CIA (n = 6) but all of the control rats that received PBS showed signs of CIA with different stages. After onset and in the course of follow up, these rats even showed progressive signs of the disease (Fig. 5). Clinical observations were then confirmed by histological analysis.

Histopathological findings. At day 21 after immunization, the rats were killed and the results were confirmed by histological examination on paw and knee joints and tissues.

Histopathological findings in rats prevented by autoreactive T cells. Acute fibrinous exudates and marked synovial thickening in the affected joint space of control rats were extensive, but no fibrinous exudates and slight or no synovial

thickening were observed in the joint space of the rats treated with autoreactive T cells. Marked and diffused tissue granulation and pannus formation was observed in the PBS group. Marked infiltration of inflammatory cells was detected in the control rats, whereas slight infiltration of inflammatory cells was detected in the rats injected with autoreactive T cells, suggesting that these T cells really prevented the inflammatory reactions around the joint.

Histopathological findings in rats treated by autoreactive T cells. Acute fibrinous exudates and marked synovial thickening in the affected joint space of controls and cases rats of groups 3 and 4 rats were extensive, but slight thickening was observed

Figure 1. A normal Albino rat

Figure 2. Albino rat with signs of CIA

Figure 3. A rat suffering from CIA before treatment with autoreactive T cells

Figure 4. A rat suffering from CIA after treatment with autoreactive T cells. Picture shows milder signs of redness and swelling

in the joint space of the cases of group 2. The signs of RA in group 1 rats were too mild to observe any change in their condition. Tissues of the cases of group 2 were less hyperemic compared to the other rats and showed less signs of hyperplasia of synovial cells. Signs of improvement and decreasing in lesions in cases of group 2 were verified through clinical evaluations of knee and paws and by pathologic investigations of stifle and tars and joints (comparing control rats). In fact, signs of relieve of disease were observed in the case group 2. Marked and diffused tissue granulation and pannus formation was observed in cases and controls in groups 3 and 4. In pannus area, severe infiltration of inflammatory cells (specially small and

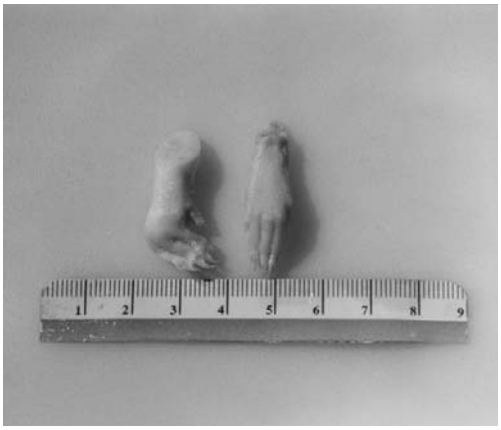

Figure 5. Comparison of the difference between the paws of rats received autoreactive T cells or PBS before injection of artheritogen substance. The right paw is belongs to a rat received autoreactive T cells before injection of artheritogen and the left paw is belongs to a rat that received PBS before injection of artheritogen. The left paw shows of swelling, inflammation and deformation

moderate sized lymphocytes), plasma cells, macrophages and fibroblasts was observed. Despite the marked infiltration of inflammatory cells in these rats, slight infiltration of inflammatory cells was detected in the cases rats in group 2, suggesting that in these rats, T cells have suppressed the inflammatory reactions around the joint (Fig. 6 and 7).

DISCUSSION

The results of this pilot study demonstrated that:
First stage: Intraperitoneal inoculation by autoreactive T cells isolated from rats with CIA, subsided the clinical and histopathological signs in rats with CIA in second stage of disease, but not rats in other stages of disease.
Second stage: Autoreactive T cells prevented the onset of disease in naive rats inoculated primarily with autoreactive T cells (although rats developed mild diseases at first). These rats were resistant against the induction of CIA. None of the control

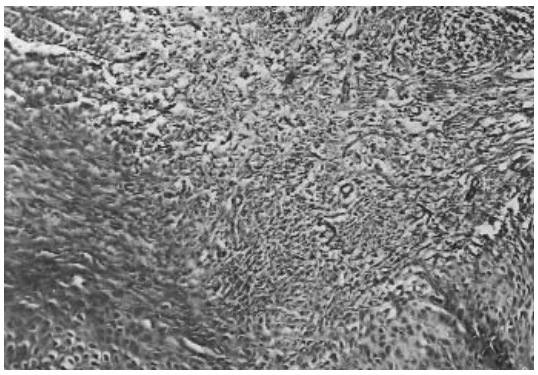

Figure 6. Pannus associated with mononuclear inflammatory cells, especially on the above and right side of the figure (H & E staining, 100X)

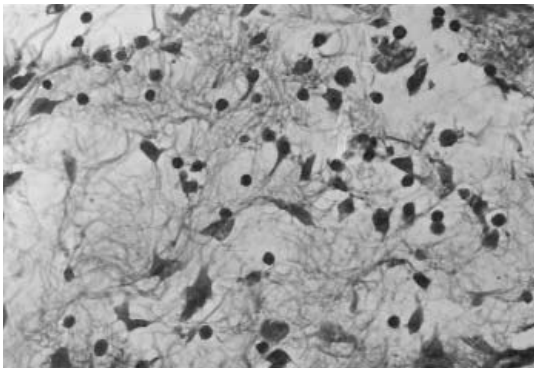

Figure 7. Pannus associated with partially severe infiltration of inflammatory cells, specially moderate and small sized lymphocytes, plasma cells, macrophages and fibroblasts (H & E staining, 400X)

rats that received PBS showed improving in the signs of CIA. It means that in our study, vaccination with autoreactive T cells prevented the subsequent induction of CIA.

Histological examination revealed an acute fibrinous exudation, synovial thickening, tissue granulation, and inflammatory cell infiltration in all the control rats inoculated with PBS, and no such inflammatory reactions were observed in rats intraperitoneally inoculated with only one injection of $1x10^5$ autoreactive T cells. The presence or absence of clinical signs of the disease corresponded with the histological evaluation of these animals.

CIA can be evoked in susceptible animals by introducing Collagen type II or activated autoreactive T cells. Because both methods ultimately results in the increase in the autoreactive cells in the recipient, in the second stage of this study we modeled the induction of autoimmunity by introducing autoreactive cells into the naive state. Initially the autoreactive cells respond vigorously, as they initiate their positive feedback loop, and reach the high levels that we interpret as autoimmune disease. During the second phase of the response, however, the regulatory cells effectively control the autoreactive cells. The autoimmune disease vanishes and the system approaches the vaccinated state. In this state, we suppose that the immune system is protected against autoimmunity because the number of regulatory cells is so high that a previously pathogenic dose of autoreactive T cells or a subsequent inoculation of an artheritogen substance can no longer induce autoimmunity (27). Activity of regulatory cells against autoreactive cells and thereby protection of rats from CIA by inoculation with autoreactive T cells, suggests a specific anti-idiotypic response (28).

Autoreactive T cells are regulated under the normal conditions. These cells play an important role in autoimmune pathogenesis when they are dysregulated because of genetic, environmental and other unknown factors associated with various autoimmune diseases. The immune regulation of autoreactive T cells may be regained by activating the regulatory network, such as the idiotype anti-idiotypic network. Immunization with autoreactive T cells (T cell vaccination) can be used as a powerful means of activating the idiotype anti-idiotypic network to deplete specific subsets of autoreactive T cells potentially involved in autoimmune conditions. It induces regulatory immune responses that closely resemble the in vivo situation, where the immune system is challenged by clonal activation and expansion of given T cell populations in various autoimmune diseases (29).

The results of our study demonstrate that CIA presentations and signs can be subsided or suppressed by autoreactive T cells. Therefore, these cells may be used as therapeutic agents in experimental autoimmunity. Furthermore, these new therapeutic approaches derived from animal models may give promises of more selective interventions for the treatment of human autoimmune diseases. Certainly, the existence of these cells warrants further investigation of the exact mechanisms responsible for the potential role of these cells in the pathogenesis and vaccination of CIA.

This study may serve as a lead for future researches on T cell vaccination in animal models. The results of this pilot study indicate that for achieving an effective T cell vaccination protocol for prevention or treatment of the experimental rheumatoid arthritis, we may need to modify and improve the protocol with a larger group of cases.

REFERENCES

1. Borghans JA, De Boer RJ. A minimal model for T-cell vaccination. *Proc R Soc Lond B Biol Sci 1995*; **259(1355)**:173-8.
2. Cohen IR. Autoimmunity to hsp 65 and the immunologic paradigm. *Adv Intern Med 1984*; **29**:147-165.
3. van Eden W, Thole JE, van der Zee R, et al. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. *Nature 1988*; **331(6152)**:171-3.
4. Zhang J, Medaer R, Stinissen P, et al. MHC-restricted depletion of human myelin basic protein-reactive T cells by T cell vaccination. *Science 1993*; **261(5127)**:1451-4.
5. Lohse AW, Bakker NP, Hermann E, et al. Induction of an anti-vaccine response by T cell vaccination in non-human primates and humans. *J Autoimmun 1993*; **6(1)**:121-30.
6. van Laar JM, Miltenburg AM, Verdonk MJ, et al. Effects of inoculation with attenuated autologous T cells in patients with rheumatoid arthritis. *J Autoimmun 1993*; **6(2)**:159-67.
7. Pearson CM. Development of arthritis, periarthritis and periostitis in rats given adjuvants. *Proc Soc Exp Biol Med 1956*; **91(1)**:95-101.
8. Pearson CM. Experimental models in rheumatoid disease. *Arthritis Rheum 1964*; **26**:80-6.
9. Holoshitz J, Naparstek Y, Ben-Nun A, et al. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. *Science1983*; **219(4580)**:56-8.
10. Holoshitz J, Matitiau A, Cohen IR. Arthritis induced in rats by cloned T lymphocytes responsive to mycobacteria but not to collagen type II. *J Clin Invest 1984*; **73(1)**:211-5.
11. Ben-Nun A, Wekerle H, Cohen IR. Vaccination against autoimmune encephalomyelitis with T-lymphocyte line cells reactive against myelin basic protein. *Nature 1981*; **292(5818)**:60-1.
12. Shapira OM, Mor E, Reshef T, et al. Prolongation of survival of rat cardiac allografts by T cell vaccination. *J Clin Invest 1993*; **91(2)**:388-90.
13. Haque MA, Yoshino S, Inada S, et al. Suppression of adjuvant arthritis in rats by induction of oral tolerance to mycobacterial 65-kDa heat shock protein. *Eur J Immunol 1996*; **26(11)**:2650-6.
14. Sharma JN, Srivastava KC, Gan EK. Suppressive effects of eugenol and ginger oil on arthritic rats. *Pharmacology 1994*; **49(5)**:314-8.
15. Karimi F. Induction of Oral Tolerance for Treatment of Experimental Rheumatoid Arthritis in Albino Rats and BALB/C Mice. PhD thesis. Faculty of Medicine, Tehran University of Medical Sciences, Iran, 1993.
16. Wood FD, Pearson CM, Tanaka A. Capacity of mycobacterial wax D and its subfractions to induce adjuvant arthritis in rats. *Int Arch Allergy Appl Immunol 1969*; **35(5)**:456-67.
17. Dubey DP, Yunis I: Manual of Clinical Laboratory Immunology. 3rd ed. 1986.
18. Wer DM: Handbook of Experimental Immunology. 3rd ed. 1978.
19. Tennant JR. Evaluation of the Trypan Blue technique for determination of cell viability. *Transplantation 1964*; **12**:685-94.
20. Bennett B. Isolation and cultivation in vitro of macrophages from various sources in the mouse. *Am J Pathol 1966*; **48(1)**:165-81.
21. Haque MA, Kimoto M, Inada S, et al. Autoreactive CD4- CD8- alpha beta T cells to vaccinate adjuvant arthritis. *Immunology 1998*; **94(4)**:536-42.
22. Wer DM: Handbook of Experimental Immunology. 3rd ed. 1978.
23. Ben-Nun A, Cohen IR. Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: process of selection of lines and characterization of the cells. *J Immunol 1982*; **129(1)**:303-8.
24. Segel LA, Jager E, Elias D, et al. A quantitative model of autoimmune disease and T-cell vaccination: does more mean less? *Immunol Today 1995*; **16(2)**:80-4.
25. Kohashi O, Aihara K, Ozawa A, et al. New model of a synthetic adjuvant, N-acetylmuramyl-L-alanyl-D-isoglutamine-induced arthritis: clinical and histologic studies in athymic nude and euthymic rats. *Lab Invest 1982*; **47(1)**:27-36.
26. Disbrey BD, Rack JH: Histological Laboratory Methods E&S. Edinburg, London: Livingston, 1970.
27. Borghans JA, De Boer RJ, Sercarz E, et al. T cell vaccination in experimental autoimmune encephalomyelitis: a mathematical model. *J Immunol 1998*; **161(3)**:1087-93.
28. Mimran A, Mor F, Carmi P, et al. DNA vaccination with CD25 protects rats from adjuvant arthritis and induces an antiergotypic response. *J Clin Invest 2004*; **113(6)**:924-32.
29. Zhang J. T-cell vaccination for autoimmune diseases: immunologic lessons and clinical experience in multiple sclerosis. *Expert Rev Vaccines 2002*; **1(3)**:285-92.