Cyclophosphamide Adjuvant Arthritis in *Trypanosoma cruzi* Infected Rats with Inflammatory Cytokine Effects

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ABSTRACT. ***Objective***. To analyze whether the cyclophosphamide (CYC) induced reestablishment of adjuvant arthritis (AA) in chronically *Trypanosoma cruzi* infected rats correlates with changes in the secretion of pro- and antiinflammatory cytokines by popliteal lymph node cells.

Methods. Inbred "l" rats infected with *T. cruzi* 90 days earlier and age matched controls were given CYC (25 mg/kg body weight) or physiologic saline 48 h before arthritis induction. Popliteal lymph node cells were collected at the time of AA induction (48 h after CYC treatment) or during the peak response, to study the concanavalin-A (ConA) or *Mycobacterium tuberculosis*-driven *in vitro* proliferation of several cytokines in their culture supernatants.

Results. Infected rats given CYC were recovered from the otherwise decreased ConA induced proliferation seen at the time of peak AA. The CYC mediated reestablishment of AA in *T. cruzi* infected rats coexisted with an increased presence of tumor necrosis factor-α in supernatants from either antigen or ConA stimulated cultures as well as interleukin 12 (IL-12) in the latter case. CYC also lowered to normal the increased IL-10 levels from ConA stimulated cultures that the *T. cruzi* group displayed at the time of inducing AA.

Conclusion. The process by which CYC restores the clinical expression of AA affects the balance between cytokines that influence the regulation of arthritis in favor of the inflammatory component. (J Rheumatol 2003;30:497–504)

Key Indexing Terms:
ADJUVANT ARTHRITIS *TRYPANOSOMA CRUZI* CYCLOPHOSPHAMIDE CYTOKINES

Infection of the mammalian host with *Trypanosoma cruzi*, the etiologic agent of Chagas' disease, is accompanied by impaired cellular immune responses to nonrelated parasite antigens[1-5]. This situation applies to our rat model of experimental *T. cruzi* infection, in which chronically infected "l" rats display a series of cellular immune abnormalities. Indeed, challenge with complete Freund's adjuvant (CFA) results in depressed adjuvant arthritis (AA). This phenomenon can be reproduced in naive recipients upon transference of spleen T cells from infected donors, before arthritis induction[6]. We found that the percentage of CD4+ cells in lymph nodes is reduced, while that of CD8+ lymphocytes and cells bearing class II MHC antigens, predominantly I-E molecules, is increased during chronic infection[7]. Extending these observations, we reported that cyclophosphamide (CYC) given in the preinductive phase of AA reestablishes the usual course of AA and prevents T cells from exerting the suppressor effect on naive recipients[8]. Immunophenotypic studies in popliteal lymph nodes at the time of inducing AA showed that rats receiving CYC 48 h earlier were recovered from the inverse balance in the major T cell subsets, and that I-E+ cells returned to normal values[9].

Rat AA is a chronic inflammatory synovitis leading to joint destruction that exhibits some similarities with rheumatoid arthritis (RA)[10]. Reports describe that T lymphocytes, in particular CD4+ cells, are required for the generation of joint damage in AA[11-13], with macrophages and their secreted cytokines also playing an important role[14]. Cytokines are major agents in the inflammatory response, as they modulate the capacity of leukocytes to generate proinflammatory mediators and also have the ability to downregulate inflammatory cells. Within this context, it can be expected that depressed AA in chronically *T. cruzi* infected rats and the CYC induced recovery of the arthritic response may be accompanied by important changes in inflammatory related cytokines. For this purpose, lymph node cells from chronically *T. cruzi* infected rats undergoing CYC treatment were collected at the time of AA induction or during the peak response to study *in vitro* the mitogen or *Mycobacterium tuberculosis*-driven proliferation and concentrations of several pro- and antiinflammatory mediators in their culture supernatants.

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Understanding the mechanisms underlying variations in the severity of AA is not only of intrinsic interest, but also essential for delineating immunointervention strategies.

MATERIALS AND METHODS

Rats and infection. Male inbred "l" rats were used in experiments. This strain derives from a cross between outbred *Rattus norvegicus* and eIIM rats (Supplement IV of the *International Survey on the Supply, Quality and Use of Laboratory Animals*, Carshalton, England, November 1964). Rats have been inbred since 1962 in our breeding facilities, and the line was in its 74th endogamous generation when experiments were done. Twenty-one-day-old rats were infected with 1×10^6 trypomastigotes of the Tulahuén strain of *T. cruzi* injected subcutaneously. Infective blood trypomastigotes were maintained by serial passage in CBi mice and obtained by cardiac puncture following ether anesthesia.

Induction of arthritis. AA was induced at 90 days postinfection by injecting 0.1 ml of CFA, containing 5 mg/ml heat killed *Mycobacterium tuberculosis* (H37Ra, Difco, Detroit, MI, USA), into the right hind footpad. In these rats, AA mostly develops in the injected limb, with early arthritic signs being noticeable 7 days after induction, and is fully established by the end of the second week postinduction. Earlier microscopic studies revealed that affected joints are heavily infiltrated by lymphocytes, macrophages, and epithelioid and giant multinucleated cells resembling a chronic granulomatous reaction[15]. Macroscopic evaluation of AA was performed at 15 days postinduction by visual scoring of individual joints, as reported[8]. The assessment was smaller joints (metatarsophalangeal and interphalangeal) 1 point; larger joints (tarsus and ankle) 2 points. The individual score was doubled if swelling was more severe than ordinarily observed. All rats were evaluated by the same operator blinded to the study groups.

Cyclophosphamide administration. Infected rats and age matched controls were inoculated intraperitoneally with a single dose of CYC (25 mg/kg body weight; Endoxan Labinca Chemical Co., Buenos Aires, Argentina) dissolved in physiologic saline, or the latter compound alone. Both treatments were given 48 h before arthritis induction. Rats receiving this CYC dose had no significant changes in their biochemical and hematological variables.

Lymph node cell proliferation assay. Infected and age matched control rats that had been given CYC or not were killed by ether anesthesia, at the time of inducing AA or at the peak of the arthritic response, that is, 48 h after CYC treatment or 15 days postinduction, respectively. Popliteal lymph nodes were then removed, disaggregated, washed twice with culture medium (RPMI 1640, Gibco), supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 5×10^{-6} M 2-mercapthoethanol, and 5% of heat inactivated fetal bovine serum (FBS) and used as a source of lymph node cells. These cells were cultured in quadruplicate in 200 µl flat bottom microtiter wells (Costar, Cambridge, MA, USA) at 2×10^5 cells/well with no stimulus or in presence of whole sonicated antigen from *M. tuberculosis* (WSA, 10 µg/ml, a gift of Dr. J. Stanford, University College, London, UK) or concanavalin-A (ConA, 2.5 µg/ml). Both stimuli were added in 100 µl of the same medium but supplemented with 10% FBS. Cultures were incubated 5 days at 37°C, 5% CO_2 in a humidified atmosphere and pulsed with [^{3}H]-thymidine for the final 18 h. [^{3}H]-thymidine incorporation was measured using a liquid scintillation counter. Results are expressed as the stimulation index (SI) calculated by dividing the mean counts of the 4 wells containing WSA or ConA by the mean counts of the 4 unstimulated control wells.

Measurements in culture supernatants. To measure the concentration of the various cytokines in supernatants of mononuclear cells stimulated with or without the mycobacterial antigen or mitogen, cultures were run in parallel and the supernatants were collected after 36 or 96 h stimulation and stored at –20°C until tested. The concentrations of tumor necrosis factor-α (TNF-α), interleukin 12 (IL-12) (in 36 h cultures), or interferon-γ (IFN-γ), IL-4, or IL-10 (in 96 h cultures) were determined by ELISA with commercial kits, as described by the manufacturers (R&D, Minneapolis, MN, USA, and Biosource, Nivelles, Belgium). Samples were assayed in duplicate and results expressed as the average of the 2 determinations. Cytokines were quantified with reference to standard curves generated using rat recombinant cytokines. The sensitivities of the assays were 10 pg/ml for IFN-γ, 5 pg/ml for IL-4, 15 pg/ml for IL-10, 4 pg/ml for TNF-α, and 5 pg/ml for IL-12 (p70 and p40 subunits). Data were presented as relative values obtained after subtracting the concentrations detected in unstimulated cultures.

Statistical analysis. Statistical comparisons were with the Kruskal-Wallis nonparametric analysis of variance and Mann-Whitney U test. The level of significance was $p < 0.05$.

RESULTS

Lymphoproliferative responses to mitogen or specific antigen stimulation. The first series of experiments compared the *in vitro* proliferation of lymph node cells in response to mitogen or mycobacterial antigen stimulation by the time AA was induced, namely, 48 h after CYC treatment. Table 1 shows a summary of one representative experiment. It can be seen that the SI toward the WSA was low, with no differences among groups. By contrast, an easily detectable proliferative response to ConA was observed in the 4 groups, particularly in those given CYC 48 h earlier, that yielded a statistically significant difference compared to their untreated counterparts.

Parallel experiments were run to further analyze the lymphoproliferation of cells obtained from regional lymph nodes at the time of fully established arthritic lesions. Mycobacterial induced blastogenesis was evident in lymph node cells, with a trend to be lower in the *T. cruzi* group that became significant when compared with the control and control plus CYC groups (Table 1). Differences appeared in the same direction when analyzing the mitogen-driven proliferation, which was significantly decreased in the *T. cruzi* group by comparison with the remaining groups. Proliferation in the control, control plus CYC, and *T. cruzi* plus CYC groups was virtually the same (Table 1). In accord with previous findings[8,9], administration of CYC to chronically *T. cruzi* infected rats 48 h before AA induction continued to reestablish a normal arthritic response (Figure 1). Control rats given CYC had a slight increase of the arthritic score, but the trend did not reach statistical significance compared with untreated controls (Figure 1).

Cytokine concentrations in culture supernatants from antigen or mitogen stimulated lymph node cells. Cytokines facilitate communication within the immune system and play a key role in physiological and immunopathological situations, such as inflammation. We analyzed the *in vitro* concentrations of cytokines, which are likely to be involved in the development of arthritic lesions. Data correspond to levels obtained upon subtracting the concentrations detected in unstimulated cultures. According to our method, the first evaluation was carried out by the time that AA was induced. IFN-γ levels in supernatants from antigen stimulated cultures were weak, showing values around the lower limit of detection, regardless of the experimental group (Figure 2A). IL-4 values in the same supernatants were also decreased, with no differences among groups (Figure 2B). As shown in Figure 2C, easily detectable concentrations of IL-10 were recorded in WSA

Table 1. Proliferative responses to mycobacterial and mitogen stimulation before induction or at the time of peak arthritis. Lymph node cells from individual rats were cultured 96 h in presence of whole sonicated antigen from *M. tuberculosis* (WSA) or concanavalin A (ConA). Data are means ± SE of the stimulation index (SI) from 4–5 rats/group (one representative of 2 independent experiments with similar results). Co+CY and Tc+Cy indicate control and *T cruzi* infected rats given cyclophosphamide (Cy), respectively. The SI for each rat was calculated by dividing the mean counts of 4 wells containing WSA or mitogen by the mean count of unstimulated control wells.

Group	Before Induction		Peak Arthritis	
	WSA	ConA	WSA	ConA
Control	1.8 ± 0.15	57 ± 4.1	3.4 ± 0.58	54 ± 5.8
Co+Cy	1.6 ± 0.37	110 ± 14*	3 ± 0.18	50 ± 3.4
T. cruzi	1.8 ± 0.05	49 ± 4.5	1.9 ± 0.1**	29 ± 0.06†
Tc+Cy	1.5 ± 0.12	103 ± 12*	2.8 ± 0.45	52 ± 4.2

*$p < 0.005$ in comparison with groups receiving no CYC treatment. **$p < 0.04$ in relation to Control and Co+Cy groups. †$p < 0.025$ in comparison with the remaining groups.

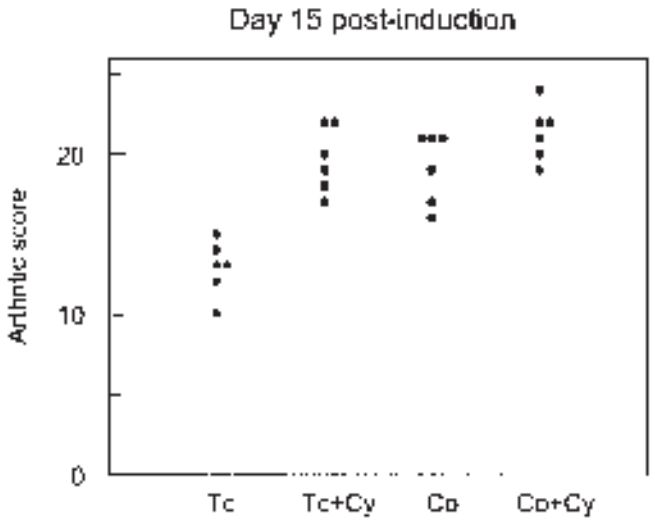

Figure 1. Effect of CYC administration on AA. Each point represents an individual result. Tc: chronically *T. cruzi* infected rats; Tc+Cy: Tc rats undergoing CYC treatment; Co: arthritic controls; Co+Cy: arthritic controls given CYC. AA was elicited following 90 days of infection, and assessed at 15 days postinduction. CYC was given intraperitoneally at a single dose of 25 mg/kg body weight, 48 h before arthritis induction. Tc statistically different from the remaining groups ($p < 0.001$).

stimulated culture supernatants, with intergroup comparisons yielding no significant differences. With regard to mitogen dependent production, abundant amounts of IFN-γ were detected in all groups, being significantly higher in uninfected rats irrespective of CYC treatment (Figure 2A). In the case of IL-4, its ConA induced production continued to be low, but was greater in rats given CYC 48 h before compared to their untreated counterparts (Figure 2B). While ConA stimulated cultures had substantial IL-10 concentrations, their levels were significantly more augmented in the *T. cruzi* group (Figure 2C).

We further proceeded to measure cytokine concentrations in culture supernatants from lymph node cells obtained at the time of peak arthritic responses. As depicted in Figure 2A and 2C, IFN-γ and IL-10 levels in either WSA or ConA stimulated cultures showed no differences among groups. IL-4 values remained as low as those recorded in nonarthritic rats, and in the case of WSA stimulated cultures, were even lower in both groups of CYC treated rats (Figure 2B).

Comparisons for each group were also made between the cytokine concentrations detected at the time of inducing (before) or during peak AA. Control and control plus CYC nonarthritic rats had a slight but significant increase of IFN-γ concentrations in their ConA stimulated cultures with respect to the arthritic counterparts ($p < 0.05$). Levels of IL-4 in ConA treated cultures from nonarthritic control plus CYC and *T. cruzi* plus CYC groups were also higher than those recorded in their arthritic counterparts ($p < 0.03$), with the latter group showing a similar but statistically insignificant trend when comparing its WSA dependent production. With the exception of WSA stimulated cultures of *T. cruzi* plus CYC rats, culture supernatants from fully arthritic rats contained significantly elevated IL-10 levels in comparison to nonarthritic animals, irrespective of the group or stimulation conditions.

Assessment of earlier synthesized cytokines. Levels of TNF-α and IL-12 were measured in culture supernatants collected at 36 h after stimulation. As shown in Figure 3A, supernatants from WSA stimulated cultures from nonarthritic rats showed no gross differences in TNF-α concentrations. Assessment in cultures subjected to ConA stimulation revealed higher TNF-α levels in both infected groups, but the trend remained insignificant (Figure 3A). Experiments in arthritic rats showed that culture supernatants from the *T. cruzi* plus CYC group contained increased TNF-α concentrations compared with their untreated counterparts regardless of whether cells were stimulated with WSA or ConA (Figure 3A). Comparisons of individual groups of values from nonarthritic and arthritic rats revealed significantly increased levels in the latter irrespective

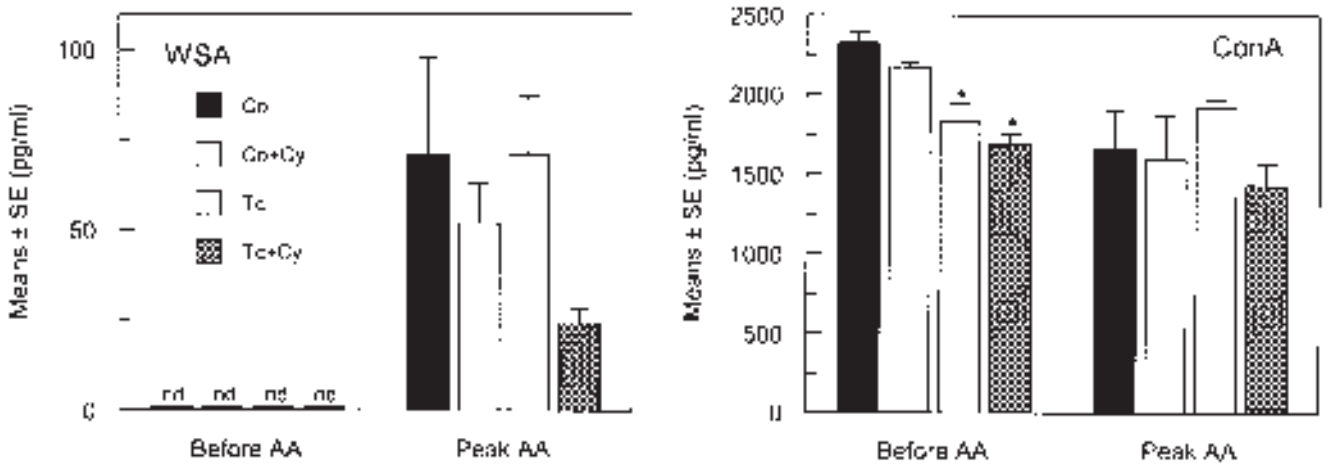

Figure 2A. Concentrations of IFN-γ in culture supernatants from arthritic and nonarthritic rats. Lymph node cells from individual rats were cultured for 96 h in presence of whole sonicated antigen from *M. tuberculosis* (WSA) or ConA. Data are means ± SE pg/ml of 4 rats/group, and illustrate one representative of 2 independent experiments. Tc: chronically *T. cruzi* infected rats; Tc+Cy: Tc rats undergoing CYC treatment; Co: arthritic controls; Co+Cy: arthritic controls given CYC; nd: nondetectable. Comparisons of groups before AA and at peak of AA; ConA stimulated cultures from Co and Co+Cy, $p < 0.05$. *Different from uninfected groups, $p < 0.01$.

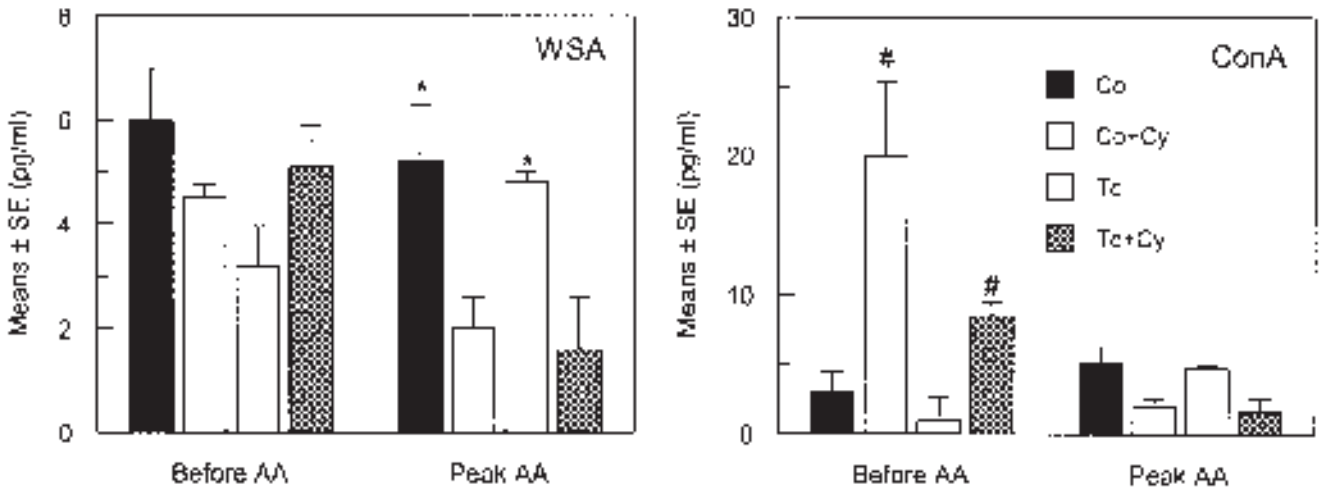

Figure 2B. Concentrations of IL-4 in culture supernatants from arthritic and nonarthritic rats. Lymph node cells from individual rats were cultured for 96 h in presence of whole sonicated antigen from *M. tuberculosis* (WSA) or ConA. Data are means ± SE pg/ml of 4 rats/group, and illustrate one representative of 2 independent experiments. Tc: chronically *T. cruzi* infected rats; Tc+Cy: Tc rats undergoing CYC treatment; Co: arthritic controls; Co+Cy: arthritic controls given CYC. Comparisons of groups before AA and at peak of AA; WSA stimulated cultures from Tc+Cy, $p > 0.05$; ConA stimulated cultures from Co+Cy and Tc+Cy, $p < 0.03$. *Different from their CYC treated counterparts, $p < 0.02$; #different from untreated counterparts, $p < 0.005$.

of whether cells were stimulated with WSA or ConA, except for the ConA stimulated cells from the *T. cruzi* group.

Culture supernatants from nonarthritic rats displayed very low amounts of IL-12, in most cases near the level of detection, irrespective of the way cells were stimulated (data not shown). Low but easily measurable levels of IL-12 were recorded in culture supernatants from arthritic rats. The *T. cruzi* plus CYC group showed a trend to increased concentrations when stimulated with WSA or ConA, statistically significant in the latter case compared to the remaining groups (Figure 3B).

DISCUSSION

As in humans[5], chronic *T. cruzi* infection in our model was found to result in depressed cell mediated immune responses affecting the development of a granulomatous reaction such as AA[6,8,15]. This abnormality does not appear to indicate a true immunosuppressed state as neither parasites nor deaths are observed during the chronic period. Instead, the phenomenon may be representative of a homeostatic mechanism to down-regulate the autoimmune component underlying the establishment of myocardial lesions[16], which these rats also displayed during chronic infection[17].

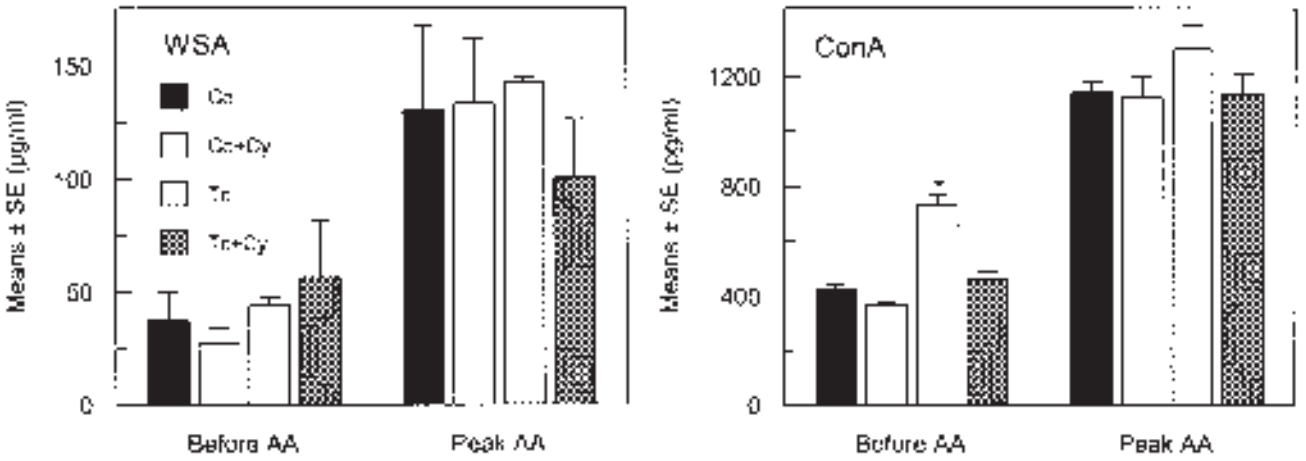

Figure 2C. Concentrations of IL-10 in culture supernatants from arthritic and nonarthritic rats. Lymph node cells from individual rats were cultured for 96 h in presence of whole sonicated antigen from *M. tuberculosis* (WSA) or ConA. Data are means ± SE pg/ml of 4 rats/group, and illustrate one representative of 2 independent experiments. Tc: chronically *T. cruzi* infected rats; Tc+Cy: Tc rats undergoing CYC treatment; Co: arthritic controls; Co+Cy: arthritic controls given CYC. Comparisons of groups before AA and at peak of AA. WSA stimulation: Co, $p = 0.05$; Co+Cy and Tc, $p < 0.015$. ConA stimulation: Tc, $p < 0.015$; remaining groups, $p < 0.01$. *Statistical difference with the remaining groups, $p < 0.008$.

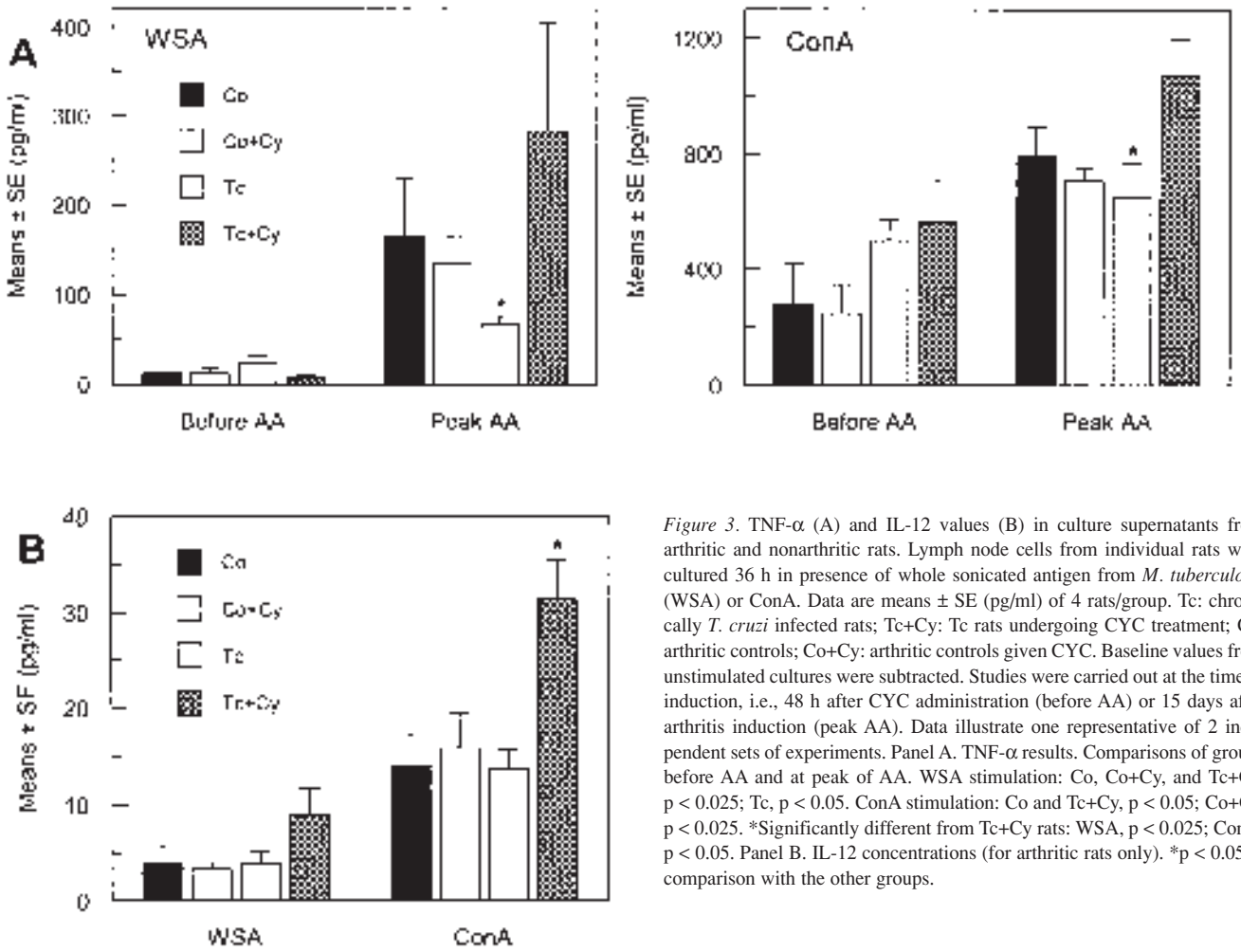

Figure 3. TNF-α (A) and IL-12 values (B) in culture supernatants from arthritic and nonarthritic rats. Lymph node cells from individual rats were cultured 36 h in presence of whole sonicated antigen from *M. tuberculosis* (WSA) or ConA. Data are means ± SE (pg/ml) of 4 rats/group. Tc: chronically *T. cruzi* infected rats; Tc+Cy: Tc rats undergoing CYC treatment; Co: arthritic controls; Co+Cy: arthritic controls given CYC. Baseline values from unstimulated cultures were subtracted. Studies were carried out at the time of induction, i.e., 48 h after CYC administration (before AA) or 15 days after arthritis induction (peak AA). Data illustrate one representative of 2 independent sets of experiments. Panel A. TNF-α results. Comparisons of groups before AA and at peak of AA. WSA stimulation: Co, Co+Cy, and Tc+Cy, $p < 0.025$; Tc, $p < 0.05$. ConA stimulation: Co and Tc+Cy, $p < 0.05$; Co+Cy, $p < 0.025$. *Significantly different from Tc+Cy rats: WSA, $p < 0.025$; ConA, $p < 0.05$. Panel B. IL-12 concentrations (for arthritic rats only). *$p < 0.05$ in comparison with the other groups.

AA is an experimental model of chronic arthritis, with T cells and their secreted products being essential in disease development[11-13]. In support of the proposal that lymph node T cells are involved in the initiation of AA reactions in joints[11,18], a recent report suggests that the synovitis occurring during AA may be caused by macrophages and lymphocytes populating popliteal lymph nodes[19]. Using lymph node mononuclear cells we found that the previously reported CYC induced recovery from AA of chronically infected rats was associated with a series of immunological changes at this level. Indeed, CYC use led to a normalized lymphoproliferation, particularly when cells were stimulated with ConA, in control and infected rats at the time of induction and in the infected group when AA was fully established. Our earlier finding of a CYC induced decrease of lymph node CD8+ T cells[9] and the report that some form of suppressor activity exists within the CD8+ subset[20] may account for these findings. Studies on T cell responses during the course of experimentally induced autoimmune diseases[21-24] identified a diversified reactivity encompassing antigens other than those initially involved in disease development. Thus, a mutual and nonexclusive explanation for the normalized mitogen-driven proliferation in the infected arthritic group is that CYC also enhances determinant spreading or diversification of T cell responses. Whatever the case, CYC-modulating effects seem to be more evident in the infected group, as CYC treated control rats showed a slight aggravation of disease severity and no increase of mitogen-driven proliferation during peak arthritis.

For insight into the mechanisms by which CYC restored AA, cytokine measurements were also carried out. Our results clearly showed that CYC lowered to normal the increased IL-10 levels of ConA stimulated cultures that the *T. cruzi* group displayed by the time the AA was induced. Additional measurements at this time point were less informative, although the low values of IL-4 were slightly increased in mitogen stimulated cultures of control and infected groups given CYC. The failure to observe an enhanced production of IFN-γ in *T. cruzi* rats receiving CYC suggests that its ability to reestablish AA is not linked to a classic pattern of Th1 immune deviation, but rather to a downregulation of IL-10 production, and perhaps IL-4 as well. It follows that chronic *T. cruzi* infection may affect immune activation in such a way that cell mediated responses create an antiinflammatory microenvironment, reducing AA development. IL-10 is a potent antiinflammatory agent that acts by inhibiting expression and synthesis of proinflammatory cytokines[25,26]. Further, treatment with recombinant IL-10 ameliorated collagen induced arthritis[27], and some studies indicate that IL-10 is likely to play a negative role in disease induction, by inhibiting both the expression of costimulatory molecules in antigen-presenting cells and IL-12 production[26]. CYC induced downregulation of IL-10 synthesis has also been described in a recent study in which a single low dose injection of CYC into tumor-bearing rats reduced the production of this cytokine[28].

Assessment at the time of fully established AA revealed no gross differences in IL-10 and IFN-γ concentrations, although IL-4 concentrations in WSA stimulated cells from both groups of CYC treated rats were even more decreased. This finding, together with results at the time of AA induction, indicate that in our model some time or antigen related involvement of Th-2 type cytokine response may be achieved by CYC treatment. Although studies in collagen induced arthritis (CIA) revealed no IL-4 expression in regional lymph nodes[29], a recent report suggests that IL-4 may be implicated in the spontaneous resolution of AA, as its expression predominated in later stages of the disease[30]. With regard to IFN-γ, this cytokine has been shown to have enhancing or inhibiting effects during the induction of or in established AA, respectively[31,32]. Our current results, however, give no support for an essential role of IFN-γ in CYC induced reestablishment of AA.

Because IL-12 and TNF-α are powerful mediators of inflammation[33,34], and may account for joint damage, we also measured these cytokines. We found consistently that CYC mediated reestablishment of AA in *T. cruzi* infected rats coincided with an increased presence of TNF-α in supernatants from either WSA or ConA stimulated cultures. Current evidence indicates that TNF-α plays a role in the pathogenesis of RA and murine models of polyarthritis[35], and is increased in joints from rats with AA[30,36,37] or regional lymph nodes from animals with CIA[29], respectively. Further, mice that overexpress TNF-α spontaneously develop arthritis[38], whereas the use of anti-TNF-α monoclonal antibodies attenuates CIA[39]. Our results are in accord with these findings and provide an additional explanation of the mechanism by which CYC reversed the suppressed AA response.

In partial agreement with results obtained by measuring TNF-α, infected rats subjected to CYC treatment also displayed increased IL-12 concentrations upon stimulating their lymph node cells with ConA. IL-12 has potent biological effects *in vitro* and *in vivo* such as induction of IFN-γ synthesis, augmentation of cytotoxic functions, and promotion of Th-1 type cytokine responses[34]. Studies in patients with RA indicate that IL-12 levels reflect disease activity and correlate with the production of proinflammatory cytokines[40]. Thus IL-12 may contribute to arthritis development by several means. That CYC normalized the otherwise increased IL-10 levels of infected rats in ConA stimulated cultures before arthritis would explain the augmented IL-12 concentrations in cultures from the arthritic counterparts, considering the inhibitory effects of IL-10 on IL-12 production[26]. Our results are in agreement with a study showing that an IL-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease[41]. According to this proposal, enhancement of disease activity is related to factors capable of decreasing IL-10 and/or increasing IL-12, in our case CYC treatment. Despite promoting Th-1 type cytokine secretion, increased IL-12 concentrations in ConA stimulated cultures from CYC treated rats were not followed by elevated IFN-γ concentra-

tions, illustrating the intricate network of cytokine regulatory influences and the ultimate immune response emerging from such interactions.

CYC is known to enhance cellular immune responses, probably by affecting the suppressor arm of immunoregulatory circuits[42,43]. Evidence in favor of CYC as altering suppressor regulatory cells in the AA model has been reported by Bersani-Amado, *et al*[44] and our earlier studies suggesting that involvement of a regulatory T cell subset together with an improved presentation of arthritogenic peptides may underlie the CYC induced enhancement of AA[9].

Extending this view, administration of CYC in a dose producing marginal immune effects in normal animals but restoring the clinical expression of AA in *T. cruzi* infected rats normalized lymphoproliferation and caused substantial changes in cytokine secretion by lymph node cells from infected rats. This evidence provides insight into the mechanisms of inhibition of the cellular immune response occurring in experimentally induced trypanosomiasis, which might mirror what happens in the human counterpart.

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