PHA - T CELLS IN RHEUMATOID ARTHRITIS : EFFECT OF DICLOFENAC SODIUM ON THE LYMPHOCYTE PROLIFERATION RATES

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SUMMARY : Rheumatoid arthritis (RA) is a disorder characterized by defective immunoregulation. Cellular immune responses are generally depressed in chronic inflammatory diseases such as rheumatoid arthritis. Investigations of cellular immune status in patients with RA showed depressed responses to phytohemagglutinin (PHA). Large amounts of PGE_2 are produced in the inflamated joints of patients with RA and supresses lymphocyte proliferation and supressor cell functions. Nonsteroidal anti inflammatory drugs (NSAIDs) inhibit the production of PGE_2 and enhance several lymphocyte functions when administered in vitro.

In this study, we evaluated the effects of a NSAID, diclofenac sodium, on PHA-induced lymphocyte proliferation. When added in vitro to cultures, diclofenac sodium did not enhance the PHA-induced proliferation rate of lymphocytes. Though the mechanism by which it evolves is related to direct inhibition of lipoxygenase with regard to the role of prostaglandings in inflammatory joint disease.

***Key Words** : Diclofenac Sodium, Lymphocytes, Rheumatoid Arthritis.*

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic, systemic inflammatory disease, predominantly affecting diarthrodial joints and frequently a variety of other organs (17). The pathologial hallmark of RA begins with a non-specific inflammation within the joint and progresses to a proliferative lesion within the synovium that can lead to joint destruction. The inflammatory lesion generates and drives the proliferative one (12). Pathogenesis of RA is characterized by autoantibodies as well as evidence of immunological hyperactivity and abnormal immunoregulation (6). Analysis of the cellular immune response in RA has identified a generalized B-cell hyperactivity and abnormalities in immunoregulatory properties of helper-T cells, inducer-T cells and monocytes. Rheumatoid factor (RF) production, polyclonal hypergammaglobulinaemia, antinuclear antibodies (ANA), synovial lymphoid follicles, lymphocytic infiltration of synovial tissue and circulating immune complexes are the laboratory findings apparent in RA (6, 13, 14, 19, 32).

In the present study, our purpose was to evaluate the lymphocyte functions in RA patients and in normal healthy subjects. The in vitro spontaneous and phytohaemagglutinin (PHA) induced proliferation rates of the lymphocytes were measured in RA patients and the results were compared with the control

group. We also studied the effects of a non-steroidal anti inflammatory drug (NSAID); diclofenac sodium on spontaneous and PHA - induced lymphocyte proliferation.

MATERIALS AND METHODS

Patient Data : Twelve seropositive (RA Latex ≥ 1 : 20) (25) RA patients (10F, 2M) who fulfilled the American Rheumatism Association (ARA) criteria (22) for definite or classical RA were studied. A group of 12 healthy subjects (10F, 2M) with no symptoms or signs of rheumatoid disease were used as normal controls. Patients seen in the rheumatology clinics were randomly selected on the basis of their willingness to participate in the study. They gave their consent forms prior to participation and the study conformed to the Decleration of Helsinki (1964) as modified by the 35th World Medical Assembly, Venice, Italy, 1983.

The mean age of the patients with RA was 47.6 years with a range of 29 to 67 years. The mean duration of disease activity was 7.33 years with a range of 1 year to 25 years. Erythrocyte sedimentation rate, determined by the standart Westergreen method, was 43 ± 31.37 mm/hr (mean ± standart deviation) in the RA patients. Patients did not take any NSAIDs for at least seven days before the blood samples were obtained. One patient was on Methotrexate, one patient was taking prednisone whereas the other one was on chloroquine. These drugs were not administered during this wash - out period.

Methods : Peripheral blood lymphocytes were isolated from heparinized blood samples of the RA patients and healthy controls. Ten mls of heparinized venous blood samples of the subjects were centrifuged at 1500 rpm for 10 minutes and the plasma was seperated (2). Medium 199 and lymphoprep were added to plasma and the mixture was centrifuged at 1700 rpm for 30 minutes. Then the lymphocyte layer was seperated and washed three times by 5ml Medium 199. To learn cell vitality, trypan blue solution (1 %) was used and cell pallets were adjusted to a concentration of $2x10^6$ cell/ml. The distrubition of the cells was done in a way that 10^5 cells were separated into 96 wells, in a triple way. In the wells where mythogenic stimulation was needed, PHA (0.01 mg/ml) was added instead of Medium 199 (20). After the cells were incubated at 37°C with 5 % CO_2 and 95 % moisture for 72 hours, 0.5 µl 3H timidine was added to cultures and incubated for another 18 hours. Finally, the cells were harvested and radioactive timidine was measured in beta counter as cpm (countper/minute) (3). All steps till the harvest period were sterilized with care. One-way Anova was used for statistical analysis.

RESULTS

In this study, spontaneous and PHA-induced proliferation rate of the peripheral blood lymphocytes, expressed in counts per minute showed a significant difference between the RA patients and normal healthy controls. In both groups PHA-induced lymphocyte proliferation was significantly elevated when compared to their initial spontaneous proliferation rates ($p<0.01$) (Table 1).

The in vitro effect of diclofenac sodium on spontaneous and PHA-stimulated lymphocyte proliferation in cultures from 12 RA patients were studied. Diclofenac sodium (50 µg/ml) did not enhance the proliferative response to PHA in cultures of lymphocytes. Moreover when diclofenac sodium was added to the cultures, we found lower spontaneous proliferation values of lymphocytes than the initial values (Table 2).

The reduction of the spontaneous proliferation rate of the lymphocytes by addition of diclofenac sodium to the culture was statistically important ($p<0.05$), but not in cultures of PHA-induced proliferation ($p>0.05$). The results of the control group showed a statistically significant difference when compared with the RA patients in both conditions (Fig 1, 2).

Counts/minute	RA patients	Healthy Controls
Spontaneous lymphocyte proliferation rate	1264.4 ± 374.75*	773.83 ± 333.74
PHA-induced lymphocyte	43089.6 ± 10826.0**	23452.0 ± 6323.22

* $p < 0.05$ versus medium of healthy controls
** $p < 0.01$ versus PHA - induced medium of healthy controls

Table 1 : PHA-stimulated lymphocyte proliferation in RA patients and healthy controls.

Counts/minute	Medium alone	Diclofenac addition (50 µg/ml)
Spontaneous lymphocyte proliferation rate	1264.4 ± 374.75	1021.2 ± 368.4*
PHA-induced lymphocyte proliferation rate	43089.6 ± 10826.0	39355.7 ± 10273.5**

* $p < 0.05$ versus medium of spontaneous lymphocyte proliferation rate
** $p > 0.05$ versus medium of PHA - induced proliferation rate

Table 2 : Effect of Diclofenac Sodium added in vitro to cultures o f lymphocytes in RA patients.

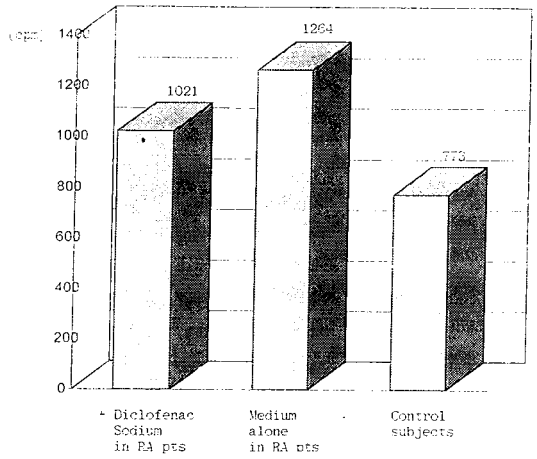

Fig - 1 : Spontaneous proliferation rates of the lymphocytes in cultures of RA patients and control subjects.

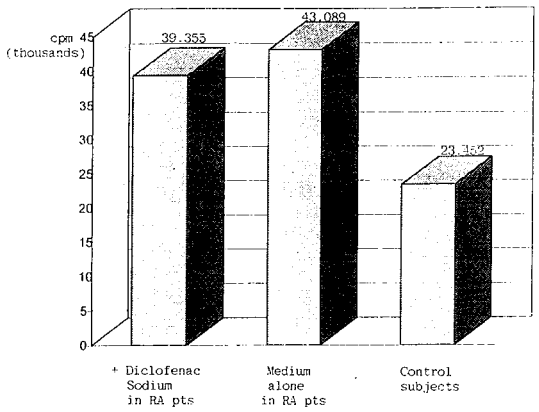

Fig - 2 : PHA - induced proliferation rates of the lymphocytes in cultures of RA patients and control subjects.

DISCUSSION

Immunologic abnormalities exist in all of the autoimmune disorders (27). Roles for both cellular and humoral immune mechanisms in the rheumatoid synovium have been proposed, and both are supported by immunopathologic findings (6). Hypergammaglobulinemia, RF production, circulating immune complexes, antikeratin and antinuclear antibodies, lymphocytic infiltration of synovial tissue are common serum abnormalities in RA (6, 13, 14, 19, 26, 31, 32).

In recent years, authors have been interested in the function of the circulating lymphocytes (4). Percy (21), found normal spontaneous lymphocyte transformation in RA patients, but a decreased response to Concanavalin A was observed by several authors (15, 16). It was concluded that in early RA of under 3 months duration, the defect was due to a decreased generation of suppressor T-cells and a decrease in B-cell response. In patients with disease activity of more than 12 months duration, T-cell response was normal, but the B-cell response continued to be deficient (23).

The response to PHA was also depressed in many studies (15, 16, 24). Menard et al (18) reported that depression of the PHA response was associated with the presence of ANA. In their study, patients existing ANA positivity showed a depressed PHA-response, whereas patients who did not have ANA had normal responses. In this study, we did not find depressed lymphocyte functions of RA patients in in vitro cultures. The spontaneous and PHA-induced proliferation rates were elevated when compared with normal controls. The correlation with ANA positivity was not investigated in our study. Disease duration of our patients ranged between 1 and 25 years. Erythrocyte sedimentation rates of 7 patients were normal and 5 patients showed elevated sedimentation rates. It's our suggestion that the chronicity and the inactivity of the disease in our patients caused the elevated proliferation rates. It has been well documented that supressor T-cell hypofunction exists in early active RA of under 3 months duration, but not in late RA of more than 6 months duration or in inactive RA (1).

It is well known that PGE_2 inhibits proliferation of T-lymphocytes, lymphocyte functions and lymphokine production (7, 11). Small amounts of PGE_2 can supress stimulation of human lymphocytes by mitogens such as PHA in vitro; and the inflammatory response is associated with the local release of arachidonic acid metabolites (29). These substances may act as negative modulators of lymphocyte function. NSAIDs inhibit the production of PGE_2 by blocking the enzyme cyclooxygenase (30). By inhibition of PGE_2, these drugs enhance several lymphocyte functions when administered in vitro. Similar effects in RA patients have previously been shown for indomethacin (8) and for piroxicam in vitro (9). It has also been demonstrated that in vitro naproxen reduced PGE_2 production in the cultures and enhanced proliferative response toward PHA in the presence of naproxen (4).

In this study, in vitro diclofenac sodium administration to the cultures depressed lymphocyte responsiveness both in spontaneous and in PHA-induced proliferation cultures. This depression was statistically significant for spontaneous proliferaton rate but unsignificant for PHA-induced proliferation rate. Prostaglandins have both inhibitory and stimulatory effects on inflammatory and immune processes (5). Prostaglandins inhibit certain cellular functions involved in the production of some forms of arthritis, suppress the inflammatory response of adjuvant arthritis and yet induce an acute inflammatory response when injected into the dog stifle joint, there remain certain paradoxes with regard to the role of prostaglandins in inflammatory joint diseases. One explanation for our conflicting results of diclofenac sodium administration to in vitro cultures might be related to paradoxal influence of prostoglandins on inflammation. It has been shown that indomethacin and other inhibitors of prostaglandin synthesis enhance cytotoxic responses against allogenic cells if added during the induction but not during the effector phase of the cytotoxic response (28).

Our results suggest that larger patient groups should be evaluated for Prostoglandin E_2 supression by in vitro cyclooxygenase inhibitors during the induction phase. Diclofenac sodium was supposed to reduce lymphocyte sensitivity to PGE_2, but our preliminary data do not support this hypothesis. Lymphocyte sensitivity to PGE_2 increases with age (10) and larger patient groups must be discussed in order to identify the altered sensitivity to PGE_2, rather than the presence of inflammation.

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