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Human post-thymic precursor cells in health and disease

IX. IMMUNOREGULATORY T CELL CIRCUITS IN PERIPHERAL BLOOD OF PATIENTS WITH RHEUMATOID ARTHRITIS

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SUMMARY

We studied T cell subpopulations and their immunoregulatory circuits in the peripheral blood of 16 patients with rheumatoid arthritis (RA) who were receiving no medications that might interfere with the results. We found normal T cells with receptors for the Fc portion of IgG or IgM as well as autologous rosette-forming T cells (T*ar* cells), a subpopulation of T cells we have found to have the properties of human post-thymic precursors. We also found that peripheral blood cells of RA patients have normal concanavalin A-induced or spontaneously-expanded suppressor cell functions. Also normal were the characteristic functions of the T*ar* cells; feedback inhibition and the generation of suppression. The normal state of these T cell subpopulations and immunoregulatory circuits in the peripheral blood of patients with RA contrasts with their various abnormalities in other connective tissue diseases. This may either mean that the immunoregulatory aberration in RA involves primarily B cells, or, if it involves T cells, that it does so primarily in the synovial membrane.

INTRODUCTION

Despite strong indications that rheumatoid arthritis (RA) is an autoimmune disease, little information has been gathered regarding its immunoregulatory processes. One obstacle to obtaining this information has been the lack of knowledge regarding the identity of the post-thymic precursor cells in man.

We have recently found that the human autologous rosette-forming T cells (T*ar* cells) have surface and functional characteristics that allowed us to postulate that they are post-thymic precursor cells (Palacios *et al.*, 1981a).

We have therefore studied T cell subpopulations including T*ar* cells and their concanavalin A (Con A) induced and spontaneously-expanded suppressor functions as well as the generation of suppression by T*ar* cells and a characteristic function of post-thymic precursor cells – feedback inhibition – in the circulating mononuclear cells of patients with RA.

MATERIALS AND METHODS

Patients. We studied 16 patients with classical or definite rheumatoid arthritis as defined by the American Rheumatism Association (Ropes *et al.*, 1958). None of the patients studied were receiving

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nor had they received for at least 1 year, corticosteroids, immunosuppressors, gold compounds or D-penicillamine. All but two of the patients were in the placebo-washout period of a study prior to starting a double-blind trial of an anti-inflammatory medication. For this, all patients had given informed consent to a protocol approved by our Institutional Committee for Human Research. The other two patients were receiving acetyl salicylic acid at doses of 4 g per day. Five patients had inactive disease and the rest had active disease as determined by the presence of more than four inflamed tender joints. Six patients had disease of recent onset (< 3 months) and the rest had had prolonged illness. We studied 16 healthy volunteers as controls who were sex- and age-matched with the RA patients.

Isolation of cells. We drew venous blood into heparinized (10 iu/ml blood) syringes, diluted it with a double volume of phosphate-buffered saline (PBS) and layered it on Ficoll–Hypaque (Pharmacia Fine Chemicals, Uppsala, and Winthrop de México, Mexico City, respectively) for gradient separation of mononuclear cells (MNC); we washed these cells three times with Hanks' balanced salt solution.

Isolation of T and B cell populations. We separated T cells from peripheral blood MNC by rosetting these with sheep erythrocytes (SRBC) at a ratio of 1 to 70. We preincubated the cells at 37°C for 15 min, centrifuged these at 1,200 r.p.m. for 10 min and then incubated them at 4°C for 18 hr. After this, we relayered the cells on Ficoll–Hypaque, centrifuged them at 1,400 r.p.m. at 4°C for 30 min, and collected separately the rosetted cells in the pellet fraction and the unrosetted cells at the interface. From the rosetted cells (total T cells) we separated the T cells with high affinity (HA-T) from those with low affinity (LA-T) for SRBC as previously described (Alarcón-Segovia, Ruíz-Arguelles & Llorente, 1979; Alarcón-Segovia & Ruíz-Arguelles, 1980). We then lysed the SRBC by hypotonic shock with water, washed the cells three times with PBS and resuspended them in minimum essential medium (MEM), enriched with 10% inactivated Ig-free fetal calf serum (Grand Island Biological Co., New York), 0·8 mg L-glutamine (Sigma Chemicals, St Louis, Missouri) and 10 μg/ml gentamicin (Schering Corporation, Kenilworth, New Jersey). HA-T cells contained 93% of cells that formed rosettes with IgM-coated ox erythrocytes (Tμ cells) and less than 1% formed rosettes with IgG-coated erythrocytes (Tγ cells).

From the unrosetted cells we obtained non-T cells by depleting these of monocytes. For this purpose we layered the cells in petri dishes and incubated the dishes at 37°C for 60 min. We recovered the non-adherent cells with Pasteur pipettes. These contained 96% B cells, identified by their surface immunoglobulins using fluorescein-tagged $F(ab')_2$ fragments of goat anti-human immunoglobulin serum (Cappel Laboratories, Cochranville, Pennsylvania), less than 2% SRBC-rosetting cells and 2% non-specific esterase-positive monocytes determined as described by Horwitz *et al.* (1977).

*Identification and purification of T*ar *cells.* We identified and isolated T*ar* cells as previously described (Palacios *et al.*, 1980). We considered as rosettes only those cells that bound three or more erythrocytes. When isolated, this population contained more than 92% T*ar* cells, less than 5% Tμ cells and less than 1% Tγ cells. All cell populations obtained showed more than 90% viability by trypan blue dye exclusion.

We identified Tγ and Tμ cells with ox erythrocyte–antibody complexes as previously described (Alarcón-Segovia & Ruíz-Arguelles, 1978; Palacios *et al.*, 1981a).

Feedback inhibition assay. We studied feedback inhibition by placing in plastic tubes 5×10^5 T cells with high affinity SRBC (having 93% Tμ cells and less than 1% Tγ cells), 1×10^6 B cells and 5×10^5 T*ar* cells, all from the same patient or normal control. We resuspended the cell mixture in MEM enriched with 10% Ig-free FCS, 0·8 mg L-glutamine, 10 μg/ml gentamicin and PWM at a final dilution of 1 : 100 in each tube. Controls included tubes with mixtures of 1×10^6 B cells, and 5×10^5 T*ar* cells without mitogen. We incubated all tubes at 37°C in a 5% CO_2, 95% air, 100% humidified atmosphere for 6 days. We determined the amount of immunoglobulin in the supernatants by mixing 0·1 ml of the supernatant with 0·2 ml of a 1 : 5 dilution of rabbit antiserum of human IgG, IgA or IgM (Behringwerke, Marburg Lahn, West Germany) in disposable plastic cuvettes with low background and reading them in a helium–neon laser nephelometer (Behringwerke) (Palacios *et al.*, 1981a, b; Alarcón-Segovia & Fishbein, 1980).

We also did ^{3}H-thymidine incorporation studies as indicators of these assays but in microtitre

tissue culture plates with flat-bottomed wells (Costar Cambridge, Massachusetts) (see Palacios *et al.*, 1981a, b). All these experiments were done in triplicate and the results are expressed as the mean.

Generation of supression by Tar cells. We studied this function as previously described (Palacios *et al.*, 1981a, b; Alarcón-Segovia & Palacios, 1980b). We did all experiments in triplicate.

Con A-induced and spontaneously-expanded suppressor function. Concanavalin A-induced suppression was studied as previously described (Alarcón-Segovia *et al.*, 1979; Alarcón-Segovia & Ruíz-Arguelles, 1980) utilizing as an indicator system both reverse haemolytic plaque-forming cells (PFC) (Alarcón-Segovia *et al.*, 1979) and secretion of immunoglobulins (Alarcón-Segovia & Fishbein, 1980).

We studied spontaneously-expanded suppression also as described before (Alarcón-Segovia *et al.*, 1979; Alarcón-Segovia & Ruíz-Arguelles, 1980) utilizing as indicator system both reverse haemolytic PFC and the amount of immunoglobulin secreted in the supernatants (Alarcón-Segovia & Fishbein, 1980).

Statistical analysis. The differences were determined by the *t*-test for paired and unpaired sample observations.

RESULTS

T cell subpopulations. All patients with rheumatoid arthritis had percentages of T*ar*, Tμ and Tγ cells that were inside those found in normal subjects. Their means were no different from those found in normal sex/age-matched controls (Fig. 1).

Suppressor cell function. Con A-induced and spontaneously-expanded suppressor cell functions were found to be normal in all 16 RA patients, both when using immunoglobulin synthesis

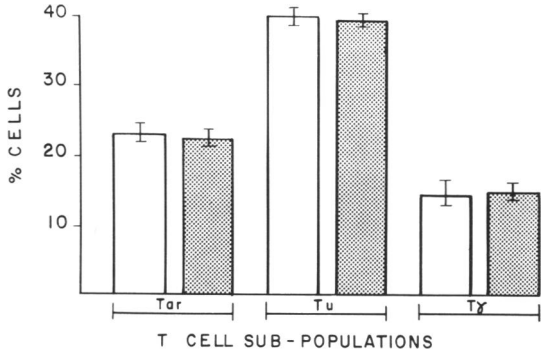

Fig. 1. T cell subpopulations in peripheral blood of patients with rheumatoid arthritis (open bars) as compared to normal, sex- and age-matched controls (shaded bars). T*ar* cells = autologous rosette-forming T cells.

determined by laser nephelometry of the supernatants or by reverse haemolytic plaque assay (PFC) as indicator systems (Table 1).

Functions of the Tar cell. Peripheral blood MNC from patients with RA behaved no differently from those of normal subjects when separated into adequate subpopulations for the study of the characteristic functions of post-thymic precursor cells. Thus, the cells from all five RA patients showed normal feedback inhibition and generation of suppressor functions (Table 2).

DISCUSSION

The normal distribution and function of T cell subpopulations in the peripheral blood of patients with RA contrasts sharply with the finding in other, and apparently closely related, connective

Table 1. Con A-induced and spontaneously-expanded suppressor function of peripheral blood MNC of patients with RA and normal controls

	Per cent suppression			
	Con A-induced		Spontaneously-expanded	
	Igs* secreted	PFC	Igs* secreted	PFC
RA	63·1 ± 4·4†	68·5 ± 3·5	62·7 ± 3·6	66·6 ± 3·6
Controls	61·8 ± 4·5	67·3 ± 4·4	63·0 ± 4·7	66·9 ± 3·1
Significance, *P* value‡	n.s.	n.s.	n.s.	n.s.

* Igs = IgG + IgA + IgM secreted into culture supernatants.
† Mean ± s.e.m.
‡ Student's *t*-test.

Table 2. Feedback inhibition function and T*ar* cell-generated suppressor function in five patients with RA

Cell assay*	^{3}H-thymidine incorporation (% suppression)	Igs synthesis (% suppression)†	Significance†
Feedback inhibition			
RA	76·8 ± 4·1§	61·4 ± 4·5	n.s.
Controls	76·0 ± 3·0	62·1 ± 2·8	n.s.
T*ar* cell-generated suppression			
RA	73·0 ± 3·4	67·0 ± 2·5	n.s.
Controls	75·0 ± 3·8	65·1 ± 3·6	n.s.

* All experiments done under PWM stimulation.
† Igs = IgG + IgA + IgM secreted into culture supernatants.
‡ Paired Student's *t*-test. n.s. = Not significant.
§ Mean ± s.e.m.

tissue diseases. Thus, SLE patients have decreased circulating Tγ cells (Alarcón-Segovia & Ruíz-Arguelles, 1978) and heterogeneously defective Con A-induced and spontaneously-expanded suppressor function (Ruíz-Arguelles *et al.*, 1980). Their circulating T*ar* cells are decreased and their functions impaired (Palacios *et al.*, 1981b). Patients with mixed connective tissue disease have decreased circulating Tγ cells and homogeneously defective Con A-induced and spontaneously-expanded suppressor functions (Alarcón-Segovia & Ruíz-Arguelles, 1980), but their T*ar* cells are increased and their immunoregulatory defect resides in Tμ signalling to T*ar* cells (Alarcón-Segovia & Palacios, 1980a). Patients with progressive systemic sclerosis have normal Tγ cells, normal suppressor function and normal T*ar* cells, both numerically and functionally, but may have decreased Tμ cells (Gupta *et al.*, 1979; Alarcón-Segovia & Palacios, 1980b) and have hyperactive helper function (Alarcón-Segovia & Palacios, 1980b). By obtaining such specific information on the immunoregulatory T cell circuits in the various connective tissue diseases we can establish their pathogenetic differences.

It is likely that an occasional patient with RA may be found to have decreased suppressor cell function (Abdou, Pascual & Racela, 1979) but this seems to be an infrequent occurrence which we did not detect in this study, in contrast to Abdou *et al.* (1979), despite the inclusion of patients with active disease of recent onset.

The finding of normal T cell circuitry in the peripheral blood cells of RA patients may indicate that their immunopathogenetic mechanism involves primarily B cells or, if it involves T cells, this

occurs mainly at a local (articular) level. This latter possibility has been approached in recent studies on the functions of synovial T cells where decreased suppressor function was found (Chattopadhyay *et al.*, 1979a, b).

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