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Antibody penetration into living cells

I. INTRANUCLEAR IMMUNOGLOBULIN IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN MIXED CONNECTIVE TISSUE DISEASE AND SYSTEMIC LUPUS ERYTHEMATOSUS

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SUMMARY

We have shown recently (Alarcón-Segovia, Ruíz-Argüelles & Fishbein, 1978) that an IgG anti-RNP antibody obtained from a patient with mixed connective tissue disease (MCTD) can penetrate viable mononuclear cells (MNC) from normal donors via their Fc receptors.

Live MNC from twelve MCTD patients incubated with goat anti-Ig antibody had intranuclear antibody with a speckled pattern in a mean of 5·5% of all MNC and 57·3% of all Fc receptor-bearing MNC. We found intranuclear immunoglobulins in all twelve patients with MCTD which were present only in cells with Fc receptors.

Only three out of twenty-one patients with systemic lupus erythematosus (SLE) were found to have intranuclear antibody in a mean of 17·2% of their Fc receptor-bearing cells. Further experiments with MNC from SLE patients revealed a partial blocking of penetration of antibody via Fc receptors.

MNC from ten scleroderma, ten rheumatoid arthritis patients and eleven normal controls did not have intranuclear immunoglobulin. *In vivo* penetration of autoantibodies into Fc receptor-bearing cells in MCTD, and probably in SLE as well, may represent an important pathogenetic mechanism.

INTRODUCTION

We have shown recently that functionally intact antibodies can penetrate living cells (Alarcón-Segovia *et al.*, 1978). For this we used an anti-RNP IgG obtained from a patient with mixed connective tissue disease (MCTD) (Sharp *et al.*, 1972). This antibody, tagged with fluorescein isothiocyanate, penetrated one fifth of all viable peripheral blood mononuclear cells (MNC) from normal donors after incubation for 1 hr at 37°C, and gave intranuclear staining with a speckled pattern characteristic of the reaction of anti-RNP with intranuclear RNP (Northway & Tan, 1972). We also showed that the antibody only enters Fc receptor-bearing cells, that blocking of the Fc receptor prevents its entrance, and that $F(ab')_2$ fragments from this antibody fail to stain nuclei from viable cells, but retain their capacity to stain the nuclei of dead cells (Alarcón-Segovia *et al.*, 1978).

In the present study we show that Fc receptor-bearing MNC from patients with MCTD carry intranuclear immunoglobulin, presumably anti-RNP antibody, *in vivo*. Occasional peripheral MNC from

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some SLE patients also have intranuclear immunoglobulin. This is not found in normal subjects nor in patients with scleroderma or rheumatoid arthritis.

MATERIALS AND METHODS

Subjects. We studied twelve patients with MCTD who fulfilled the criteria proposed for this disease which include the presence of high titres of anti-RNP antibodies (Alarcón-Segovia, 1976); twenty-one patients with systemic lupus erythematosus (SLE) all of whom fulfilled the preliminary criteria for the classification of SLE of the American Rheumatism Association (Cohen *et al.*, 1971); ten patients with classical or definite rheumatoid arthritis (RA) (Ropes *et al.*, 1959); ten patients with scleroderma, eight with progressive systemic sclerosis, one with the CRST syndrome (Schimke, Kirkpatrick & Delp, 1967) and one with linear scleroderma. Eleven normal subjects served as controls.

Methods. We drew blood from all subjects in the morning, while fasting, into both syringes containing 5 u of heparin per ml of blood and non-heparinized syringes. Mononuclear cells were separated immediately from the heparinized blood by the Ficoll–Hypaque gradient method of Böyum (1968). Over 95% of the MNC obtained were viable as judged by Trypan blue exclusion. We incubated the MNC with fluorescein isothiocyanate-labelled goat antibody to human immunoglobulins (Behringwerke AG, Marburg-Lahn, Germany) at the optimum time and temperature for anti-RNP antibody penetration into viable cells (Alarcón-Segovia *et al.*, 1978). Cell viability was controlled throughout the study by Trypan blue exclusion. At least 200 cells were counted in each study. Lymphocyte receptors for sheep red blood cells (SRBC) were determined by their ability to form rosettes with unsensitized SRBC, according to the method of Frøland (1972). Readings were taken after 18 hr incubation at 4°C. An active subpopulation of lymphocytes bearing receptors for SRBC, characterized by the formation of early rosettes, was determined as described previously (Rivero *et al.*, 1977).

Lymphocytes bearing receptors for the Fc fragment of IgG were determined by the formation of rosettes with three or more chicken erythrocytes coated with anti-chicken erythrocyte IgG raised in rabbits and prepared in our laboratory (Samarut, Brochier & Revillard, 1976; Díaz-Jouanen *et al.*, 1977). Lymphocytes bearing receptors for C3 were determined by their ability to form rosettes with three or more SRBC coated with the IgM fraction of commercial anti-SRBC rabbit serum (Difco Laboratories, Detroit, Lot 599330), and complement derived from fresh human serum (Díaz-Jouanen *et al.*, 1977; Stjeinswärd *et al.*, 1972).

Antibodies to extractable nuclear antigen (ENA) were determined by the method of Sharp *et al.* (1971). Ribonuclease treatment of the ENA allowed anti-RNP antibody to be distinguished from other ribonuclease resistant antigens present in the ENA, particularly Sm antigen (Sharp *et al.*, 1971). Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on frozen sections of rat stomach and kidney, as well as by complement fixation and counterimmunoelectrophoresis using calf thymus antigens as described previously (Alarcón-Segovia & Fishbein, 1970; Alarcón-Segovia, Fishbein & Estrada-Parra, 1975a). Rheumatoid factor was determined by the method of Singer & Plotz (1956). C3 serum levels were determined by radial immunodiffusion in commercial antibody–agar plates (Behringwerke AG, Marburg-Lahn, Germany). Serum DNA binding activity was studied by the Farr method, modified by Lewkonia *et al.* (1977).

RESULTS

Mixed connective tissue disease

Fluorescent studies with viable cells. All twelve patients with MCTD had viable peripheral blood MNC containing intranuclear antibody, as shown by the speckled immunofluorescent staining of their nuclei with goat antibody to human immunoglobulins. This nuclear staining was clearly distinguishable from membrane and cytoplasmic fluorescent staining (Fig. 1), and was observed in both lymphocytes and monocytes as determined morphologically. The percentage of viable MNC with fluorescence stained nuclei ranged from 1–11% (Table 1) (mean$\pm$s.e.m. 5·46$\pm$0·95%). This percentage was lower than that of viable MNC capable of forming Fc rosettes with antibody-coated chicken erythrocytes, which ranged from 6–12% in individual MCTD patients, with a mean of 9·46$\pm$0·70%. We found intranuclear Ig in 57·3% of the MNC-bearing Fc receptors. Conversely, all cells shown to have intranuclear antibody *in vivo*, as detected by staining with the anti-human IgG antiserum, were found to form Fc rosettes in preparations in which MNC were incubated simultaneously with antibody-coated chicken erythrocytes and fluorescent anti-immunoglobulin antibody, while also being checked for viability by Trypan blue exclusion (Fig. 1). In these combined preparations we found that 68% of all Fc rosette-forming cells from MCTD patients had intranuclear Ig. This differs from normal MNC incubated with fluorescent anti-RNP antibody, in which all Fc rosette-forming cells develop intranuclear fluorescence (Alarcón-Segovia *et al.*, 1976).

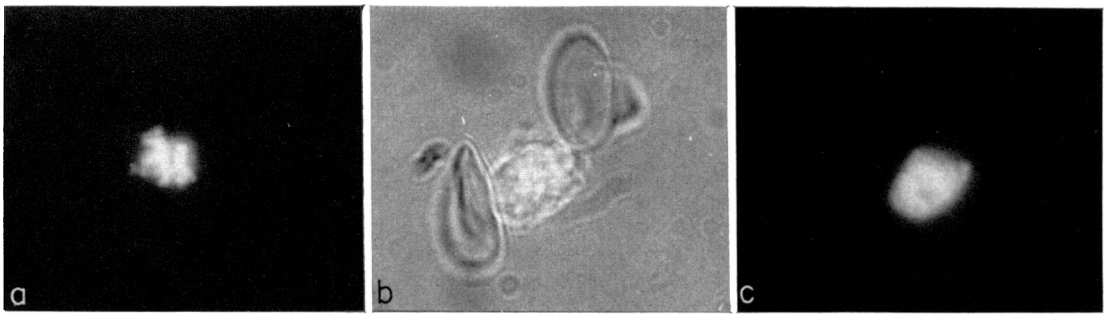

FIG. 1. (a) Intranuclear speckled staining with fluorescent anti-Ig of a circulating viable mononuclear cell from a patient with MCTD. (b) Fc rosette of a viable lymphocyte with antibody-coated chicken erythrocytes. The core cell excluded Trypan blue and shows intranuclear staining with anti-Ig, photographed under simultaneous epifluorescent and bright field illumination. (c) The same Fc rosette seen under epifluorescence shows the intranuclear fluorescent staining. Epifluorescent microscopy was done with Zeiss filters UG 1/3, Ft. 420, LP 418 and photographed with Tri-X-pan Kodak film at an original magnification × 800.

Patients with MCTD were found to have significantly higher percentages of MNC showing intracytoplasmic ($P<0{\cdot}005$) or membrane ($P<0{\cdot}005$) fluorescence, after incubation with the anti-human immunoglobulin antibody, than normal subjects (Table 2). MCTD patients differed from normal subjects in that they had intracytoplasmic Ig in both lymphocytes and monocytes, while in normal subjects intracytoplasmic fluorescent staining was limited to cells with the morphological characteristics of monocytes. Patients with MCTD also had significantly lower percentages of Fc rosette-forming MNC than normal controls ($P<0{\cdot}01$).

Results of sequential studies in four patients with MCTD may be seen in Table 3.

Clinical and laboratory correlations. There was no correlation between the log serum titre of anti-RNP antibody and the percentage of MNC that had demonstrable intranuclear antibody *in vivo*. Two patients with MCTD who had had high serum titres of anti-RNP antibody at various other times but in whom the antibody was not detectable in their serum by haemagglutination assay at the time of this study (Table 1), were both found to have intranuclear antibody in their circulating MNC with a speckled immunofluorescent pattern. One of these two patients (C.O.) had ANA in her serum detectable by indirect immunofluorescence on rat kidney sections (grade + of + + + +), where a speckled pattern was also noticed. The other patient (A.C.), with negative anti-RNP antibody, also had negative serum fluorescent ANA. Only 1% of the total MNC from this patient had detectable intranuclear immunoglobulin. However, there were other MCTD patients with similarly low percentages of intranuclear antibody-bearing MNC who were found to have high anti-RNP antibody titres.

Analyses of the percentages of viable MNC with detectable intranuclear antibody, of cells with the various lymphocyte receptors studied, of the anti-lymphocytic and ANA, serum DNA binding activity, rheumatoid factor, C3 levels and of the corticosteroid dosages showed no correlations.

Systemic lupus erythematosus

Only three of the twenty-one SLE patients were found to have detectable intranuclear Ig in their circulating MNC (Table 4), despite the fact that all but one of them had serum ANA detectable at the time of the study by at least one method. The three SLE patients had detectable antibody in very small percentages of their MNC (1, 1 and 2%, respectively). Immunofluorescent, receptor and antibody findings in these three patients are shown in Table 4. Apart from the presence of intranuclear Ig, they did not differ from other SLE patients with regard to clinical manifestations or laboratory findings. All three were among the eleven SLE patients studied who were not receiving corticosteroids.

MNC from SLE patients showed little cytoplasmic fluorescence, and the proportions of cells having it were similar to those found in normal controls. Conversely, MNC from SLE patients had significantly higher percentages of membrane fluorescence as compared with the controls ($P<0{\cdot}0005$).

TABLE 1. Intranuclear antibody in viable MNC from patients with MCTD. Relationship to Fc receptor-bearing cells, antibodies to ENA and DNA binding activity

Patient	Nuclear fluorescence percentage of viable cells	Percentage of Fc receptor-bearing MNC	Percentage of Fc receptor-bearing MNC with nuclear stain	Antibody to ENA, titre		Percentage of DNA binding*
				No RNAse treatment	RNAse-treated antigen	
D.J.	9	11	81·8	1:12800	0	72·17
D.D.	11	12	91·6	1:1600	0	44·98
R.P.	3	7	42·8	1:65536000	0	20·83
A.M.	9	14	64·2	1:8192000	0	60·40
A.A.	8	12	66·6	1:1600	0	13·07
C.M.	7	8	87·5	1:16384000	0	0·39
A.B.	7	7	100·0	1:16384000	0	25·06
A.C.	2	10	20·0	1:4096000	0	32·85
J.J.	4	6	66·6	1:400†	0	42·83
C.O.	6	9	66·6	0†	0	32·80
A.C.	1	7	14·2	0†	0	59·98
L.L.	1	11	9·0	1:25600	1:3200‡	63·25

* Normal: < 36·0%.
† Strongly positive at various other times.
‡ Negative at various other times.

TABLE 2. Summary of findings of intracellular Ig, presence of antibodies to ENA and DNA binding in patients with various connective tissue diseases and in normal controls

	Percentage fluorescence location			Percentage of Fc receptor-bearing MNC	Percentage of Fc receptor-bearing MNC with nuclear fluorescence	Percentage of antibody titre to ENA ≥ 1:400	Percentage of DNA binding*
	Nuclear	Cytoplasmic	Peripheral				
Mixed connective tissue disease (12)†	5·46±0·95‡	3·3±0·95	12·23±1·34	9·46±0·70	57·31±8·8	84·62	39·00±6·39
Systemic lupus erythematosus (21)	0·19±0·11	0·38±0·16	12·28±0·77	8·23±0·55	2·45±1·34	14·28	61·93±5·49
Scleroderma (10)	0	3·3±0·47	6·4±0·77	8·7±0·77	0	0	23·99±7·70
Rheumatoid arthritis (10)	0	1·8±0·95	7·3±1·40	7·8±0·98	0	10	36·43±7·16
Normal controls (11)	0	0·27±0·13	7·18±0·70	12·72±0·90	0	0	24·45±3·70

* Normal: < 36%.

† Parentheses indicate number of subjects in each group.

‡ Mean ± s.e.m.

TABLE 3. Results of sequential studies of intranuclear antibodies in four patients with MCTD. Relationship to Fc receptors and anti-ENA antibody

Patient	Date	Percentage of cells bearing nuclear fluorescence	Percentage of cells bearing Fc receptor	Percentage of Fc rosette-forming cells bearing nuclear fluorescence	Antibody to ENA, titre	
					No RNAse treatment	RNAse-treated antigen
A.C.	8.3.77	2	10	20.0	1:4096000	Neg.
	8.24.77	4	7	57·1	1:4096000	Neg.
	9.7.77	3	8	37·5	1:25600	1:200
C.M.	5.17.77	7	8	87·5	—*	—
	6.13.77	4	9	44·4	1:16384000	Neg.
	9.14.77	5	10	50·0	1:800	Neg.
A.A.	5.19.77	8	12	66·6	1:600	Neg.
	8.1.77	4	11	36·3	1:8192000	Neg.
	8.30.77	0	8	0	—	—
R.P.	6.7.77	2	7	28·5	—	—
	9.14.77	3	8	42·8	1:65536000	Neg.

* Not done.

TABLE 4. Intranuclear antibody in viable MNC from three SLE patients. Relationships to Fc receptor-bearing cells, antibodies to ENA and DNA-binding activity

Patient	Percentage of MNC bearing nuclear fluorescence	Percentage of Fc receptor-bearing MNC	Percentage of Fc receptor-bearing MNC with nuclear fluorescence	Antibody to ENA, titre		Percentage of DNA binding*
				No RNAse treatment	RNAse-treated antigen	
R.C.C.	1	6	16·66	Neg.	Neg.	39·65
M.S.	1	6	16·66	Neg.	Neg.	95·96
S.M.	2	11	18·18	1:200	1:200	79·75

* Normal: < 36·0%.

So as to discount the possibility that serum factors present in SLE could interfere with cell penetration of the antibody, we carried out three sets of experiments in selected SLE patients. In one set of four experiments we washed MNC from SLE patients extensively, checked them for viability and fixed them on microscope slides with formalin–ethanol. Slides were stained with the goat anti-human immunoglobulin antibody. These experiments were carried out to circumvent the requirement of Fc receptors for the anti-Ig antibody to enter viable cells (Alarcón-Segovia *et al.*, 1978). Extensive washing with control of viability immediately prior to fixation ruled out the possibility that any intracellular antibody found had entered after cell death. In a second set of sixteen experiments, we incubated MNC from SLE patients at 37°C in a 5% CO_2 atmosphere for 24 hr to allow the shedding of any proteins attached to the cell surface that might have been blocking the Fc receptors (Messner, Kennedy & Jelinik, 1975). We then washed the cells, checked their viability and incubated them with the anti-immunoglobulin antibody. Finally, in another set of three experiments, MNC from lupus patients were treated with neuraminidase in an attempt to uncover hidden receptors (Keder, Ortiz de Landazuri & Fahey, 1974) prior to incubation with the anti-human immunoglobulin antiserum. None of these manipulations increased detection of intranuclear antibody in the MNC of SLE patients.

TABLE 5. Intranuclear antibody in viable MNC from SLE patients following incubation with fluorescein-labelled anti-RNP or anti-human immunoglobulins antibodies

	Percentage of MNC bearing intranuclear fluorescence	
Patient	Anti-RNP antibody	Anti-human Igs antibody
E.R.	3	0
F.M.	3	0
R.R.	0	0
M.S.	1	1
C.O.	0	0
G.F.	4	0
E.V.	4	0
A.L.	3	0
R.A.	0	0

To determine whether the Fc receptor-bearing MNC from SLE patients were penetrated by an antibody already known to enter normal MNC, we carried out a number of experiments in which we incubated MNC from SLE patients with a fluorescein-labelled anti-RNP IgG previously shown to enter viable cells (Alarcón-Segovia *et al.*, 1978). The results obtained are presented in Table 5. This antibody entered the nucleus of up to 4% of viable MNC from six out of nine SLE patients. This was still less than findings in normal MNC incubated in the anti-RNP antibody where all subjects showed antibody penetration to the nucleus of nearly 20% of the cells (Alarcón-Segovia *et al.*, 1978).

Scleroderma

None of the ten patients with scleroderma had intranuclear Ig in their circulating MNC, despite the presence of serum ANA in half of them, as shown by at least one of the methods used for their detection. Cytoplasmic fluorescence after incubation with the anti-Ig serum was, however, significantly increased in scleroderma patients as compared to normal controls ($P<0{\cdot}005$) and it was observed in both lymphocytes and monocytes. The percentages of cells showing membrane fluorescence were similar to those found in normal controls.

Rheumatoid arthritis

None of the patients with rheumatoid arthritis had intranuclear Ig detectable in their MNC. Cytoplasmic fluorescent staining was found in significantly higher proportions of their MNC than in normal controls ($P<0{\cdot}01$). However, practically all MNC from RA patients showing cytoplasmic fluorescence were monocytes. Membrane fluorescence in MNC from RA patients did not differ from normal.

Controls

None of the normal controls had intranuclear Ig detectable by immunofluorescence.

DISCUSSION

The finding of intranuclear antibody in viable circulating mononuclear cells from patients with MCTD correlates well with our previous finding that an anti-RNP antibody obtained from a patient with MCTD is capable of penetrating normal live MNC incubated within it (Alarcón-Segovia *et al.*, 1978). Furthermore, it shows that after one antibody penetrates the cell up to the nucleus, a second antibody may do likewise, apparently also by penetrating the cell through Fc receptors, as suggested by the observation that all MNC from MCTD patients shown to bear intranuclear antibody are capable of forming Fc rosettes. The fact that this second antibody also gives a speckled pattern indicates that the intranuclear Ig with which it reacts in MNC from MCTD patients, is in turn reacting with one of the nuclear antigens with such nuclear distribution, most likely RNP.

Because the antibody used to detect the intranuclear immunoglobulin was not human, our findings also demonstrate that antibodies can penetrate living cells from other species, at least in the circumstances involved in this study.

The difference found in MCTD between the percentages of Fc receptor-bearing cells and those of cells giving intranuclear staining with the anti-immunoglobulin antibody could be due to blocking, in some of the Fc receptor-bearing cells, by the first antibody to enter the cell, since we have shown previously that all normal Fc receptor-bearing cells incubated for 1 hr with a fluorescein-tagged anti-RNP antibody are themselves penetrated by it (Alarcón-Segovia *et al.*, 1978). Blocking of the entry of a second antibody by the first antibody to enter the cell could take place at various levels along the pathway of the antibody from the Fc receptor to the nucleus. In a limited intracellular space, a second antibody might not have the easy access of the first antibody to the antigenic sites, or the first antibody to enter the cell may have exhausted, albeit transitorily, the energetic transport mechanisms, along the route.

The finding of higher percentages of viable MNC bearing intracytoplasmic and/or membrane immunoglobulin in MCTD may indicate a backlog in the penetration of antibody all the way to the nucleus, caused by the untagged antibody already present along this pathway.

Antibody penetration into living cells has long been considered an unlikely phenomenon. Scant evidence that it might occur has included the finding that antinucleoside antibodies may interfere with the development of fertilized sea urchin eggs (Rosenkranz *et al.*, 1964), or inhibit the culture growth of Chinese hamster lung cells transformed by methylcholanthrene (Liebeskind *et al.*, 1971). Anti-RNA antibodies have been found to inhibit the replication of virus-infected mouse fibroblasts (Varesio, Capuccinelli & Forni, 1975). An occasional biopsy from patients with MCTD or SLE has been found to give intranuclear fluorescent staining in direct immunofluorescent studies with anti-IgG antiserum (Svec, Blair & Kaplan, 1967; Tan & Kunkel, 1966; Paronetto & Koffler, 1965; Shu *et al.*, 1977; Gilliam & Prystowsky, 1977). Patients showing this phenomenon in their biopsies are usually found to have antibodies to extractable nuclear antigens, whether RNAse sensitive (RNP) or not (Sm antigen) (Shu *et al.*, 1977; Gilliam & Prystowsky, 1977). The fact that the immunoglobulin-bearing cells found in such biopsies are not primarily MNC, and are thus unlikely to bear Fc receptors, might indicate that the antibody did not penetrate them while alive or intact. However, cells other than MNC have been found to bear Fc receptors (Hopf, Meyer-zum-Büschenfelde & Dierich, 1976; Guyer, Koshland & Knopf, 1976;

Borthistle *et al.*, 1977), or may develop them as a result of viral infection (Rahman *et al.*, 1976; Watkins, 1964). If anti-RNP antibody penetrated skin cells while alive and did it by means of their Fc receptors, the possibility arises that in MCTD, and perhaps in SLE as well, some cells carry a virus capable of eliciting surface Fc receptors in cells other than MNC.

Several factors may favour the penetration of antibodies to RNP into viable cells or its detection: their frequently high concentration, their homogeneous nature, their predominantly IgG class and the peculiar speckled pattern they provide in fluorescent studies upon reaction with their nuclear antigen. The paucity of intranuclear Ig-bearing viable MNC in SLE patients found in this study, despite the presence of ANA in their serum, may in turn reflect their heterogeneity in both antigenic specificity and immunoglobulin class (Alarcón-Segovia *et al.*, 1970), which could result in a single antibody rarely achieving enough concentration intranuclearly to yield a detectable pattern. The simultaneous presence of cytophilic antibodies (Bluestein & Zvaifler, 1976) that could interfere with antibody penetration, and/or the decrease in numbers of Fc receptor-bearing cells which occurs in SLE (Díaz-Jouranen *et al.*, 1977) could also be contributory. Despite the fact that MNC from SLE patients were found to have increased membrane fluorescence after incubation with the anti-immunoglobulin antiserum, experiments in which MNC were incubated to allow shedding of membrane-bound antibodies (Messner *et al.*, 1975), or were killed to circumvent the requirement for Fc receptors for antibody entry (Alarcón-Segovia *et al.*, 1978) did not yield an increase in the detection of intranuclear fluorescence. However, when SLE MNC were incubated with the anti-RNP antibody, which we found previously to penetrate readily into all Fc receptor-bearing cells, the antibody was observed to penetrate into more cells than had been found to show intranuclear staining with anti-immunoglobulin antibody, but into fewer cells than those having Fc receptors. These findings indicate that the low incidence of intranuclear Ig-bearing MNC in SLE may be due to both the amount and heterogeneity of the antinuclear antibodies these patients have, as well as to blocking of the staining antibody.

Some Fc receptors have been found to be sensitive to cortisone (Borthistle *et al.*, 1977; Gupta Good, & 1977) which might explain the decreased penetration of antibody in some of the patients studied who were receiving corticosteroids. In addition, the continued presence of immune complexes may have caused the irreversible dissociation of Fc receptors as shown by Cordier, Samarut & Revillard (1977), thus preventing antibody penetration. This could also explain the decrease in Fc receptor-bearing MNC found in the various connective tissue diseases.

Mononuclear cells from patients with scleroderma were found to have increased intracytoplasmic fluorescence which was not limited to monocytes but was also present in lymphocytes, suggesting that it is not only the result of phagocytosis. An uracil-specific anti-RNA antibody has been found in the serum of patients with scleroderma which, although likely react preferentially with nucleolar RNA, could react with cytoplasmic RNA as well (Alarcón-Segovia *et al.*, 1975b; Alarcón-Segovia & Fishbein, 1975). Conversely, the intracytoplasmic fluorescence found in rheumatoid arthritis was virtually limited to monocytes and probably represented mainly phagocytosis.

Antibody penetration of cells, which occurs *in vivo* in MCTD and probably in SLE as well, may be an important pathogenetic mechanism in these two diseases. Antibodies to nucleic acids, nucleo histones and acidic nuclear proteins could, upon entering the cell and reacting with their corresponding antigen, alter their functions. The selective penetration of these antibodies into Fc receptor-bearing cells could contribute or even cause the abnormality of the modulation of the immune response which is paramount in these two diseases.

We have recently found that antibody to RNP can specifically penetrate T cells bearing Fc receptors for IgG and cause their deletion (Alarcón-Segovia & Ruíz-Argüelles, 1979). This is important because these T cells have been found to have suppressor activity on B cells (Moretta *et al.*, 1977).

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