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Macrophage Fcγ Receptors Expression Is Altered by Treatment with Dopaminergic Drugs[1]

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Macrophage Fcγ receptors have an important role in host defense and the pathophysiology of immune mediated disorders. Alteration of splenic macrophage Fcγ receptors expression predisposes to severe infection. Inhibition or blockade of splenic macrophage Fcγ receptors is one of the mechanisms by which immune cytopenias improve. Dopaminergic drugs have clinically significant regulatory functions on the immune response. Using an experimental model in the guinea pig we assessed the effect of commonly used dopaminergic drugs on the expression of macrophage Fcγ receptors. Three dopa-antagonists, bromocryptine, leuprolide, and pergolide, and seven dopa-antagonists, chlorpromazine, SCH 23390, metochlopramide, sulpiride, veralipride, alizapride, and cisapride, were studied. Following guinea pig treatment with dopaminergic drugs, the clearance of IgG-sensitized RBCs *in vivo*, the *in vitro* binding of IgG-sensitized RBCs by isolated splenic macrophages and flow cytometry with monoclonal antibodies were performed. Treatment with dopa-agonists enhanced the clearance of IgG-sensitized RBCs, the *in vitro* binding of IgG-sensitized RBCs by isolated splenic macrophages, and the cell surface expression of both macrophage Fcγ receptors, and vice versa, dopa-antagonists impaired macrophage Fcγ receptors expression. Macrophage FcγR1,2 was more sensitive than FcγR2 to such dopaminergic effect. These alterations of macrophage Fcγ receptors expression are mediated by both D1 and D2 dopamine receptors, with a major participation of D2 receptors. Dopaminergic drugs alter the clearance of IgG-coated cells by an effect at the expression of splenic macrophage Fcγ receptors.

Key Words: **Fcγ receptors; macrophage; dopaminergic drugs.**

INTRODUCTION

Splenic macrophage Fcγ receptors have a critical role in host defense. Patients with end-stage renal disease (1) or cirrhosis (2) have an altered function of their splenic macrophage Fcγ receptors which predisposes them to severe infections. Splenic macrophage Fcγ receptors are also involved in the pathophysiology of immune mediated disorders by their role in the clearance of immune complexes (3–7). Inhibition (8, 9) or blockade (10) of splenic macrophage Fcγ receptors is one of the mechanisms by which immune cytopenias improve.

Dopaminergic agents have immunoregulatory actions. Treatment with bromocryptine, a dopa-agonist that inhibits prolactin (PRL) secretion, ameliorates autoimmune diseases and contributes to the treatment of graft rejection (11, 12). Chlorpromazine is a dopa-antagonist of the phenothiazine family that has been associated with autoimmune diseases with a preponderant humoral autoimmune component (13–18). In addition, chlorpromazine inhibits delayed-type hypersensitivity responses (19) and prevents lethal endotoxinemia in mice (20). We have previously observed that veralipride, a dopa-antagonist, inhibits macrophage Fcγ receptors expression (21).

Using an experimental model in the guinea pig (3, 4, 8, 9) we studied the effect of the administration of dopaminergic drugs on splenic macrophage Fcγ receptors expression as potential therapeutic agents in immune cytopenias and immune complex diseases. Dopa-agonists increased splenic macrophage Fcγ receptors expression, whereas dopa-antagonists impaired splenic macrophage Fcγ receptors expression. The effect of dopaminergic drugs on macrophage Fcγ receptors depends on both D1 and D2 dopamine receptors, with a predominant D2 receptor participation. Dopaminergic drugs altered the function of both guinea pig macrophage Fcγ receptor classes. FcγR1,2 was more sensitive to such dopaminergic action than the other guinea pig macrophage Fcγ receptor, FcγR2.

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METHODS

All studies were performed with 500- to 600-g male Duncan–Hartley guinea pigs obtained from Criffa, Barcelona, Spain. Animals were injected with equal volumes of a solution of dopaminergic drugs in PBS as previously reported (22, 23). Sham controls received 1 ml of PBS not containing dopaminergic drugs. All animals were injected sc in the dorsal neck fat pad every afternoon for 7 consecutive days and studied on the day after the last injection. Chlorpromazine (Clp) was obtained from Sigma Chemical Co. and SCH 23390 from Research Biochemicals Inc. (Natick, MA). Metochlopramide (M), sulpiride (S), veralipride (V), alizapride (A), cisapride (Cs), bromocryptine (B), leuprolide (L), and pergolide (P) were obtained from the pharmacy. Rabbit IgG anti-guinea pig RBC antibodies were prepared as previously described and isolated by Sephacryl S-300 gel filtration and QAE ion exchange chromatography (Pharmacia, Piscataway, NJ), and were free of IgM as determined by Ouchterlony analysis and SDS–PAGE (3, 4, 8, 9).

Clearance of IgG-coated erythrocytes. Blood was drawn from anesthetized guinea pigs by cardiac puncture. Washed erythrocytes were radiolabeled with ^{51}Cr-sodium chromate (Amersham, Madrid, Spain) and sensitized with an equal volume of IgG antibody, so as to be coated with approximately 800 IgG molecules per erythrocyte as described (3, 4, 8, 9). Animals treated with dopaminergic drugs or PBS control for 7 days were injected intravenously with 1.7×10^8 ^{51}Cr-labeled cells. Samples of blood were obtained 5, 15, 30, 60, 90, and 120 min after injection and cell-associated radioactivity was measured in a γ-counter (Gamma 8000, Beckman Instruments, Inc., Fullerton, CA). Studies were also performed with heat-altered erythrocytes to investigate splenic trapping mediated by nonimmune mechanisms (3, 4, 8, 9). Clearance curves were plotted by expressing the number of blood counts per minute at each time point as a percentage of the number of counts per minute at 5 min. Clearance at time 60, 90, and 120 min was analyzed to calculate a *P* value for the difference between control and experimental clearance curves using Student's *t* test. In addition, for each day's clearance study, the percentage inhibition of clearance (mean ± SEM) above control was calculated at 90 and 120 min according to the formula

$$\text{Percentage inhibition} = 100 \times [1 - (\text{cpm}_c - \text{cpm}_x)/(\text{cpm}_c - \text{cpm}_{ea})],$$

where cpm_c refers to counts per minute for the untreated control animal injected with unsensitized cells, cpm_x to the experimental animal treated with dopaminergic drugs and injected with IgG-coated erythrocytes, and cpm_{ea} to control animals treated with PBS only (no dopaminergic drug) and injected with the control IgG-sensitized erythrocytes. A negative value for percentage inhibition indicates enhancement of clearance. This formula compares treated animals with the control animals studied on the same experimental day and expresses the data as percentage alteration of clearance, where 100% inhibition of clearance by dopaminergic drugs corresponds to the situation in which the clearance of IgG-sensitized erythrocytes (cpm_x) is identical to that of unsensitized erythrocytes (cpm_c) (8, 9).

Binding of IgG-coated erythrocytes by splenic macrophages in vitro. Guinea pigs were sacrificed, splenectomy was performed immediately, and the spleens were placed in RPMI 1640 + 10% heat-inactivated fetal calf serum + glutamine (complete RPMI). Fine suspensions of spleen cells in PBS with no Ca^{++} or Mg^{++} were obtained using tissue grinding sieves with a 100-μm mesh, followed by Nytex gauze sieving (50 μm, Tetko Inc., Elmsford, NY). The cells were then layered onto a discontinous Percoll (Pharmacia, Inc., Barcelona, Spain) gradient (35–70%) and spun at 600*g* and 22°C for 30 min. Mononuclear cells were recovered from the intermediate layer (45–55% Percoll). The cells were then washed at room temperature with PBS containing 5mM EDTA + 10% heat-inactivated FCS previously dialyzed with PBS without Ca^{++} or Mg^{++} and resuspended to 2×10^6 cells/ml in complete RPMI. Cells were incubated for 60 min at 37°C in a 5% CO_2 atmosphere in 75-cm^2 tissue culture flasks to allow the macrophages to adhere. Nonadherent cells were removed with complete RPMI, and adherent cells were mechanically dislodged after 10 min of incubation at 37°C in a 5% CO_2 atmosphere with PBS containing 5 mM EDTA + 10% dialyzed heat-inactivated FCS. Cells were washed once and resuspended in complete RPMI. More than 95% of the resultant cells were viable mononuclear cells as determined by their ability to exclude trypan blue, and >90% of cells ingested latex beads and were stained with nonspecific sterase. Monolayers of adherent cells were prepared as previously described by incubating 1×10^6 cells on a glass coverslip in a 35-mm plastic petri dish at 37°C for 45 min under 5% CO_2. More than 95% of the cells were adherent to glass (8, 9). For experiments using Fcγ receptor activity *in vitro*, guinea pig erythrocytes were sensitized with 500 molecules of IgG per erythrocyte as described above, and 1 ml of erythrocytes (5×10^7 cells/ml) was incubated with the macrophage monolayers at 37°C under 5% CO_2 for 20 min. The monolayers were washed, air-dried, and stained with Wright–Giemsa, and 200 consecutive macrophages were inspected under oil im-

mersion for the number of erythrocytes bound per cells (8, 9). The number of macrophages which bound more than three erythrocytes was then determined. No internalization of erythrocytes was observed under the above experimental conditions. Treatment with dopaminergic drugs had no effect on the yield or viability of mononuclear cells isolated from the spleen.

Flow cytometry. Monoclonal antibodies with specificity against guinea pig macrophage FcγR1,2 (VIA2 IgG1) and FcγR2 (VIIA1 IgG1) (24) were utilized in indirect immunofluorescence binding studies to assess Fcγ receptor cell surface expression. These monoclonal antibodies were the generous gift of Drs. Yamashita and Nakamura, Sapporo, Japan. Cells (5×10^5) were incubated with saturating concentrations of each monoclonal antibody for 60 min at 4°C and washed twice with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin and 0.02% sodium azide. To measure bound antibody, a FITC-labeled goat anti-mouse antibody (Tago, Inc., Burlingame, CA) was added for 30 min at 4°C. The cells were again washed twice and fixed with 4% paraformaldehyde. Cell-associated fluorescence was measured using a FACSCAN cytometer with Consort-32 software (Becton–Dickinson, Madrid, Spain). For all samples 10,000 events were recorded on a logarithmic fluorescence scale, and the actual mean fluorescence intensity (MFI) for each sample was determined using the Consort-32 software. In order to correct for autofluorescence, the MFI of a nonreactive murine IgG1 antibody (M3) was subtracted from the MFI of the anti-FcγR1,2- and anti-FcγR2-stained cells. Percentage of change in fluorescence intensity was calculated by

$$\%\ \text{change} = [(\text{MFI of anti-Fc}\gamma\text{R-treated cells} - \text{MFI of M3-treated cells})/(\text{MFI of anti-Fc}\gamma\text{R-untreated cells} - \text{MFI of M3-untreated cells})]^{-1} \times 100.$$

To demonstrate the specificity of dopaminergic drugs on macrophage Fcγ receptors expression, we included an additional control with an irrelevant guinea pig pan-macrophage surface antigen, GPB (Seralab Ltd., Sussex, England). Treatment with dopaminergic drugs did not influence the cell surface expression of this pan-macrophage antigen while altering the expression of both macrophage Fcγ receptors, FcγR1,2 and FcγR2.

Effect of in vivo dopa-agonists and dopa-antagonists on membrane mobility of FcγR1,2 and FcγR2. Immunofluorescence capping experiments were performed to examine any possible effects of *in vivo* administered dopaminergic drugs on the membrane mobility of FcγR1,2 and FcγR2. Splenic macrophages (5×10^5) from guinea pigs treated with either 25 mg/kg chlorpromazine, 50 mg/kg veralipride, 25 mg/kg SCH 23390, veralipride (50 mg/kg) plus SCH 23390 (25 mg/kg) for 7 days or, from sham-treated animals, were incubated with saturating concentrations of monoclonal antibodies for 30 min at 0°C on ice. After two washes at 0°C in PBS/0.5% BSA without sodium azide, FITC-labeled goat anti-mouse antibody was added as in the flow cytometry experiments. Cells were incubated at either 0 or 37°C for 20 min, washed, fixed in paraformaldehyde, and spun onto microscope slides in a centrifuge. Several hundred cells per slide were examined under epifluorescence with a fluorescent microscope (Carl Zeiss, Oberkochen, Germany), data not shown.

Serum prolactin levels. Animals were treated with different doses of dopaminergic drugs as described above. Serum samples were taken at baseline and each day 2 h after the injection of dopaminergic drugs or PBS (sham) and were frozen at −80°C in 200-μL aliquots until prolactin levels were determined by ELISA (Atom, Madrid, Spain).

RESULTS

Macrophage Fcγ receptors are critical in host defense and the pathologic process of immune disorders (1–10). The pharmacologic modulation of macrophage Fcγ receptors expression opens the possibility of preventing infections and treating immune mediated conditions. Dopaminergic drugs alter immune functions and are sometimes associated with overt clinical manifestations (11–21). We studied the function of splenic macrophage Fcγ receptors in guinea pigs treated with dopaminergic agents by assessing the clearance of IgG-sensitized erythrocytes *in vivo* (Fig. 1), the *in vitro* binding of IgG-sensitized erythrocytes by isolated splenic macrophages (Fig. 2 and Table 1), and the cell surface expression of both guinea pig macrophage Fcγ receptor classes, FcγR1,2 and FcγR2, using flow cytometry with specific monoclonal antibodies (Table 2).

Sham-treated animals cleared 50 ± 3% of the IgG-sensitized erythrocytes at 120 min (Fig. 1). Nonimmune erythrocyte clearance (i.e., not Fcγ receptors dependent) was determined by examining the survival of heat-damaged erythrocytes not coated with IgG. More than 95% of the heat-damaged erythrocytes were cleared, while 97 ± 3% of the unsensitized RBCs were still circulating at 120 min (Fig. 1) for either shams or animals treated with dopaminergic drugs. Treatment with dopa-agonists: B, L, P significantly enhanced the clearance of IgG-sensitized erythrocytes in more than 88% of the animals (Fig. 1). Clearance curves in ani-

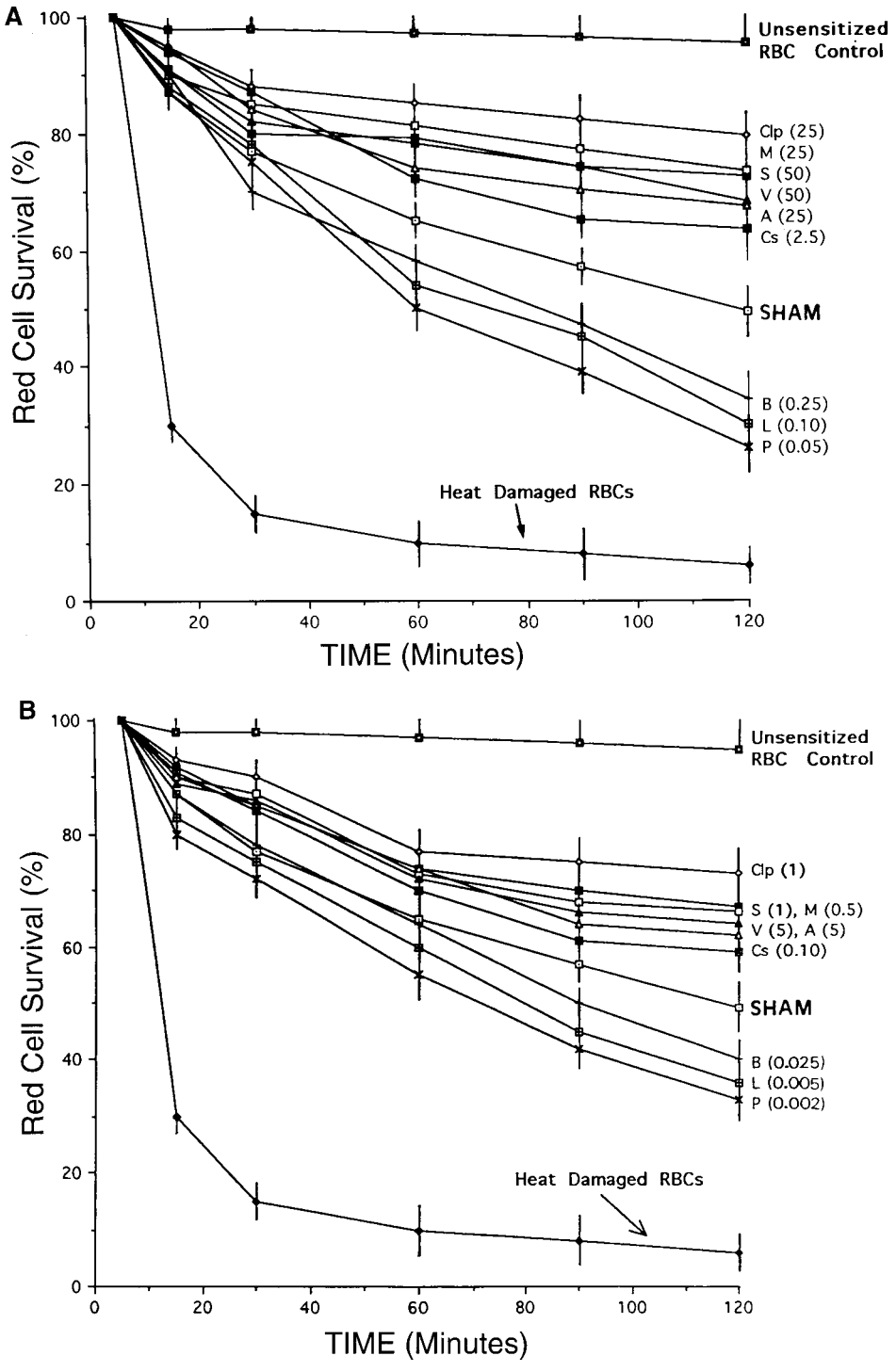


FIG. 1. Clearance of IgG-sensitized RBCs in guinea pigs treated with high (A) and lowest effective doses (B) of dopaminergic drugs. The dose-dependent effect of dopa-agonists bromocryptine (B), leuprolide (L), and pergolide (P) and dopa-antagonists chlorpromazine (Clp), metochlopramide (M), sulpiride (S), veralipride (V), alizapride (A), and cisapride (Cs) over sham controls is shown. Numbers in parentheses indicate the selected dose of dopaminergic agent presented. Red cell survival is expressed as percent ^{51}Cr-labeled IgG-sensitized RBCs ($x \pm$ SEM) remaining in the circulation at each indicated time point. Survival of unsensitized RBCs and that of heat-damaged RBCs was not different between sham controls and animals treated with dopaminergic drugs.

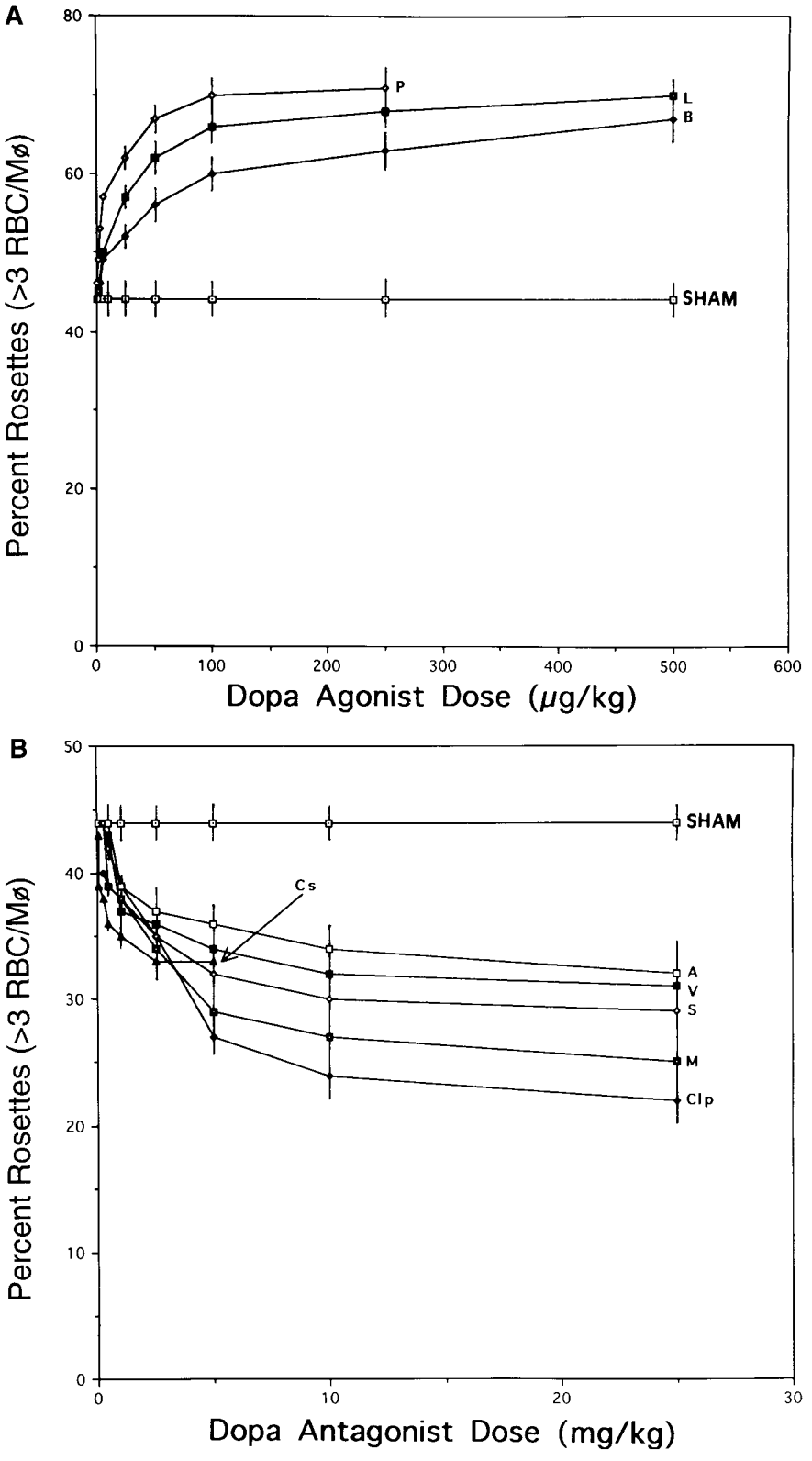


FIG. 2. Alteration of the *in vitro* binding of IgG-sensitized erythrocytes by splenic macrophages isolated from guinea pigs treated for 7 days with dopa-agonists (A) and dopa-antagonists (B) at the indicated doses. The percent isolated splenic macrophages binding more than three IgG-sensitized RBCs ($x \pm$ SEM) in treated animals and sham controls is indicated.

TABLE 1

Alteration of the *in Vitro* Binding of IgG-Sensitized RBCs by Isolated Splenic Macrophages after Treatment with Dopaminergic Drugs

Dopaminergic drugs	Dose	% Macrophages binding > three IgG-sensitized RBCs ($x \pm$ SEM)
Dopa-agonists	(μg/kg)	
Bromocryptine (B)	0.005	50 ± 1
	0.050	56 ± 2
	0.250	62 ± 3
	0.500	66 ± 3
Leuprolide (L)	0.005	50 ± 2†
	0.025	57 ± 2
	0.050	62 ± 3
	0.500	68 ± 3
Pergolide (P)	0.002	48 ± 2*
	0.005	54 ± 3
	0.025	62 ± 3
	0.250	71 ± 3
Sham		44 ± 2
Dopa-antagonists	(mg/kg)	
Chlorpromazine (Clp)	1	39 ± 1*
	2.5	37 ± 1
	10	33 ± 2
	25	22 ± 2
Metochlopramide (M)	0.5	39 ± 1*
	2.5	34 ± 2
	5	28 ± 2
	25	25 ± 3
Sulpiride (S)	1	39 ± 1*
	5	32 ± 2
	10	30 ± 2
	25	25 ± 3
Veralipride (V)	1	37 ± 1†
	5	34 ± 2
	10	32 ± 2
	25	31 ± 3
Alizapride (A)	2.5	37 ± 2*
	5	36 ± 2
	10	35 ± 2
	25	33 ± 3
Cisapride (Cs)	0.1	38 ± 1*
	0.5	36 ± 1*
	1	35 ± 1
	10	33 ± 3

Note. Splenic macrophages were isolated from animals treated for 7 days with dopaminergic drugs at the indicated dose. The percent splenic macrophage binding more than three IgG-sensitized RBCs ($x \pm$ SEM) over sham controls is indicated as an index of *in vitro* macrophage Fcγ receptors function. $P < 0.001$, unless indicated otherwise (*$P < 0.01$, †$P < 0.05$).

mals treated with B, L, and P at high (Fig. 1A) and low doses (Fig. 1B) are shown. The percentage of IgG-sensitized RBCs remaining in circulation after 120 min in animals treated with B, L, and P is indicated. No consistent enhancement of clearance of IgG-sensitized erythrocytes was observed at doses of B, L, and P below those indicated in Fig. 1B.

To assess the effect of dopa-antagonists on macrophage Fcγ receptor function, we chose a mixed D1/D2 dopa-antagonist, Clp, a D1 receptor antagonist, SCH 23390, and five D2 receptor antagonists, M, S, V, A, and cisapride Cs. Treatment with Clp, M, S, V, A, or Cs (Fig. 1) significantly impaired the clearance of IgG-sensitized erythrocytes in more than 85% of the animals. The lowest effective dose of dopa-antagonists impairing the clearance of IgG-sensitized cells is indicated in Fig. 1B.

Fcγ receptor function was determined *in vitro* by the percentage of isolated macrophage binding more than three IgG-sensitized erythrocytes (Fig. 2 and Table 1). The percentage of splenic macrophage isolated from animals treated with dopa-agonists B, L, or P binding more than three IgG-sensitized erythrocytes was significantly higher than that of macrophages from sham controls, 44 ± 2%, and vice versa, splenic macrophages isolated from animals treated with dopa-antagonists Clp, M, S, V, A, and Cs bound significantly less IgG-sensitized erythrocytes than those from sham controls (Fig. 2 and Table 1). No consistent alteration of binding of IgG-sensitized erythrocytes was observed with doses of dopaminergic agents under those indicated in Table 1.

We further studied the effect of treatment with dopaminergic agents on the splenic macrophage Fcγ receptors cell surface expression using flow cytometry with specific monoclonal antibodies against those receptors (Table 2). Guinea pig macrophages express two types of Fcγ receptors, FcγR1,2 and FcγR2 (24). Treatment with dopa-agonists B, L, or P increased the expression of both splenic macrophage Fcγ receptor classes, FcγR1,2 and FcγR2, whereas dopa-antagonists decreased Fcγ receptors expression. Macrophage FcγR1,2 was more sensitive to dopaminergic modulatory signals than the other macrophage Fcγ receptor, FcγR2. The lowest effective dose of dopaminergic drugs altering Fcγ receptors expression is indicated in Table 2. Immunofluorescence capping experiments were performed in order to examine any possible effects of *in vivo* administered dopaminergic drugs on the membrane mobility of FcγR1,2 and FcγR2. We consider whether the lipophilic dopaminergic drugs might alter the mobility of surface membrane receptors, thus contributing to the observed effects on FcγR1,2 and FcγR2 expression. To this end *in vitro* capping experiments were performed comparing splenic macrophages isolated from treated animals to those from sham controls. Bromocryptine (0.025 and 0.250 mg/kg), chlorpromazine (1, 5, and 25 mg/kg), veralipride (5, 25, and 50 mg/kg), and SCH 23390 (5 and 25 mg/kg), which were found to be the most potent agents in studies described above, were chosen. Cells incubated at 0°C to

TABLE 2

Alteration of Fcγ Receptors Mean Fluorescence Intensity by Treatment with Dopaminergic Drugs

Dopaminergic drugs	Dose	% Alteration of mean fluorescence intensity: FcγR1,2 ($x \pm$ SEM)	% Alteration of mean fluorescence intensity: FcγR2 ($x \pm$ SEM)
Dopa-agonists	(μg/kg)		
Bromocryptine (B)	0.005†	12 ± 1	10 ± 1
	0.025	19 ± 1	16 ± 1
	0.250	36 ± 2	28 ± 2
	0.500	41 ± 2	30 ± 2
Leuprolide (L)	0.005*	14 ± 1	12 ± 1
	0.025	21 ± 1	16 ± 1
	0.250	44 ± 2	32 ± 2
	0.500	48 ± 2	34 ± 2
Pergolide (P)	0.001	17 ± 1*	13 ± 1†
	0.025	26 ± 1	19 ± 1
	0.100	46 ± 2	34 ± 2
	0.250	53 ± 2	41 ± 2
Dopa-antagonists	(mg/kg)		
Chlorpromazine (Clp)	0.500	−22 ± 2	−19 ± 1
	10	−46 ± 2	−32 ± 1
	25	−64 ± 4	−47 ± 3
	50	−65 ± 4	−50 ± 4
Metochlopramide	0.500	−20 ± 2	−18 ± 1
	10	−42 ± 2	−30 ± 1
	25	−59 ± 4	−50 ± 3
	50	−60 ± 4	−52 ± 4
Sulpiride (S)	1	−18 ± 1	−16 ± 1*
	5	−29 ± 2	−25 ± 1
	25	−40 ± 3	−35 ± 3
	50	−58 ± 4	−49 ± 4
Veralipride (V)	1	−17 ± 1	−14 ± 1*
	5	−26 ± 2	−20 ± 1
	25	−47 ± 3	−35 ± 3
	50	−51 ± 3	−42 ± 3
Alizapride (A)	1	−17 ± 1	−11 ± 1†
	5	−42 ± 2	−30 ± 1
	25	−49 ± 3	−38 ± 3
	50	−53 ± 3	−43 ± 3
Cisapride (Cs)	0.100	−15 ± 1*	−11 ± 1†
	0.500	−24 ± 2	−19 ± 1
	2.500	−32 ± 2	−26 ± 2
	5	−45 ± 33	−31 ± 2

Note. Splenic macrophages were isolated from animals treated for 7 days with the indicated daily doses of dopaminergic drugs and the cell surface expression of both splenic macrophage Fcγ receptor classes, FcγR1,2 and FcγR2, was studied by flow cytometry with monoclonal antibodies. Results are expressed as percent alteration ($x \pm$ SEM) of the mean fluorescence intensity compared to that of sham controls. Positive values indicate enhanced cell surface expression, while negative values represent inhibition of cell surface expression. $P < 0.001$, unless indicated otherwise (*$P < 0.01$, †$P < 0.05$).

prevent membrane movement showed a uniform diffuse ring pattern when stained for either FcγR1,2 or FcγR2. When incubated at 37°C, the majority of cells displayed aggregates or patches of membrane fluorescence, with some cells showing an intense polar distribution of staining for both Fcγ receptors, similar to that reported for the ligand-induced capping of lymphocyte surface immunoglobulin (34). No significant differences were observed between shams and animals treated with dopaminergic drugs for either FcγR1,2 or FcγR2 staining intensity or distribution. Dopaminergic drugs do not have a major effect on the membrane mobility of these receptors.

Chlorpromazine has a predominant D1 and D2 dopa-antagonist action, while benzamides (metochlopramide, sulpiride, veralipride, alizapride, and cisapride) have a more selective D2 dopa-antagonist effect (27–32). A selective D1 dopa-antagonist would help to differentiate the relative contribution of D1 and D2 dopa-antagonists action on the expression of macrophage Fcγ receptors. We used the selective D1 dopa-antagonist, SCH 23390, to assess the contribution of dopamine receptors D1 and D2 on the observed alterations of macrophage Fcγ receptors expression. In doing so, animals were treated with chlorpromazine (mixed D1/D2-antagonist), SCH 23390 (D1-antagonist), veralipride (D2-antagonist), or SCH 23390 plus veralipride (D1 + D2 dopa-antagonists). Treatment with chlorpromazine, SCH 23390, or veralipride significantly decreased the clearance of IgG-sensitized erythrocytes (Fig. 3A). A D1-antagonist, SCH 2390, significantly decreased the clearance of IgG-sensitized erythrocytes; at 120 min 60 ± 2% of the IgG-sensitized erythrocytes remained in the circulation in 25 mg/kg treated animals compared to 50 ± 3% for sham controls ($P < 0.001$). Nevertheless, the inhibition of the clearance of IgG-sensitized erythrocytes by SCH 23390 (D1-antagonist) is not as intense as that induced by veralipride (D2-antagonist) or chlorpromazine (D1/D2 antagonist).

Treatment with a D1-antagonist, SCH 23390 (25 mg/kg), plus a D2-antagonist, veralipride (50 mg/kg), does not significantly decrease the clearance of IgG-sensitized erythrocytes, compared with either V (50 mg/kg), Clp (50 mg/kg), or SCH 23390 (25 mg/kg). A specific D1-antagonist (SCH 23390) does not further decrease the clearance of IgG-sensitized erythrocytes induced by D2 dopa receptor-antagonists. Therefore, dopa-antagonists impaire the clearance of IgG-sensitized erythrocytes through a main effect at D2 dopa receptor level. These findings were confirmed by the *in vitro* binding of IgG-sensitized erythrocytes and the cell surface expression of both guinea pig Fcγ receptors by splenic macrophages isolated from animals treated with dopa-antagonists (Fig. 3B). Treatment with chlorpromazine, veralipride, or SCH 23390 significantly decreased the percentage of macrophages binding more than three IgG-sensitized erythrocytes. The lowest effective dose inhibiting the binding of IgG-sensitized

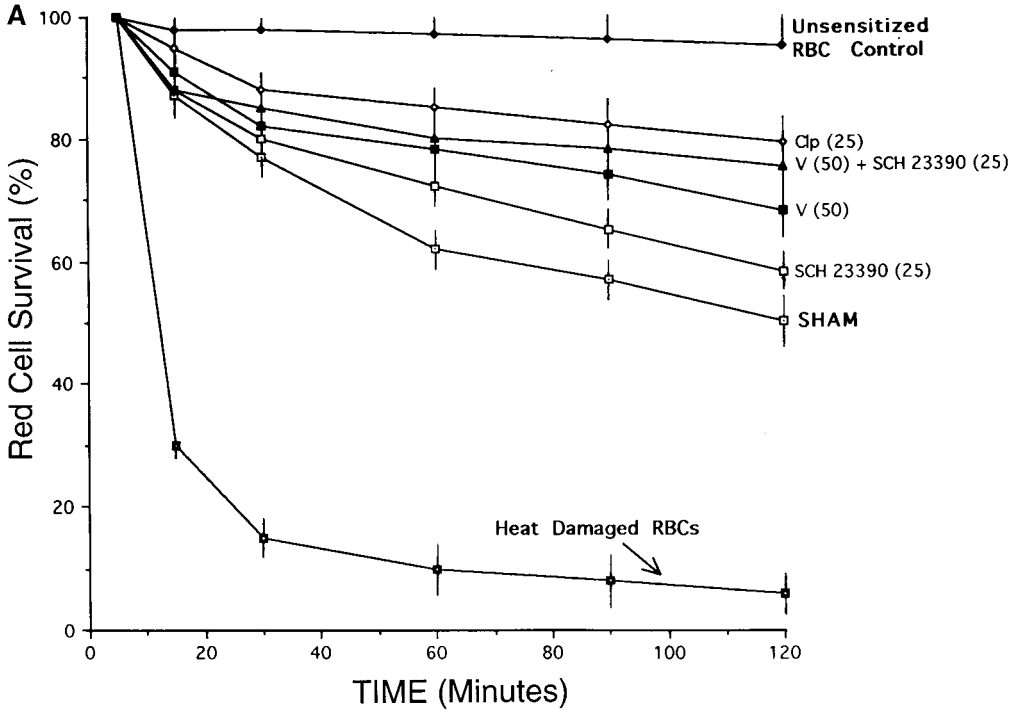


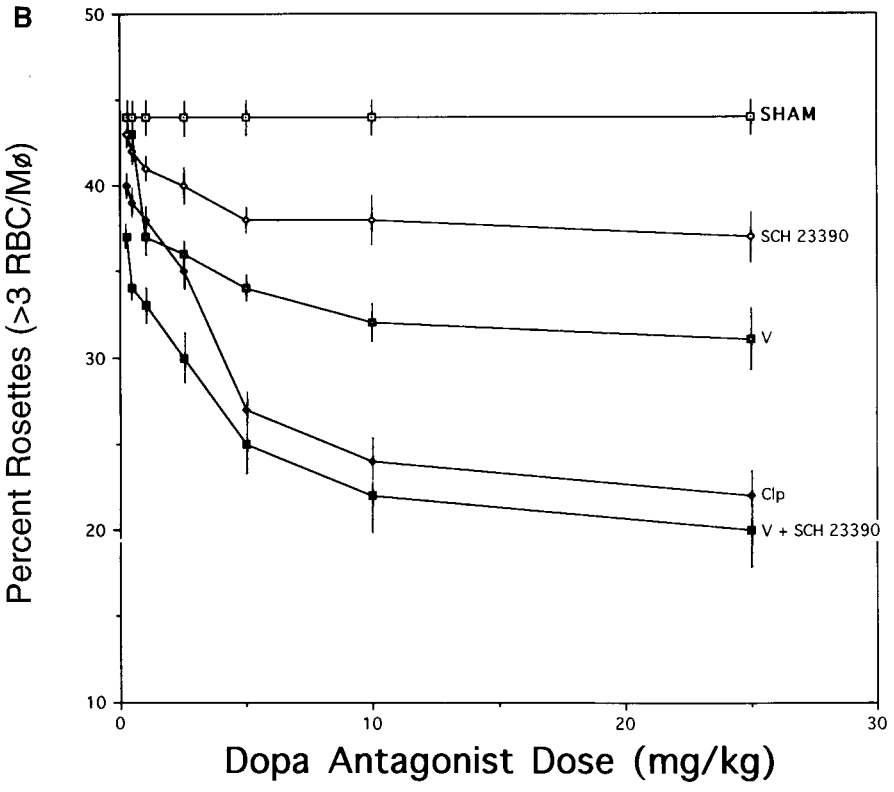
FIG. 3. Effects of treatment with a mixed D1/D2 dopa-antagonist (Clp), a D1 dopa-antagonist (SCH 23390), a D2 dopa-antagonist (V), and a D1 dopa-antagonist (SCH 23390) plus a D2 dopa-antagonist (V) on macrophage Fcγ receptors expression. A shows the inhibition of clearance of IgG-sensitized RBCs in guinea pigs treated with dopa-antagonists at the indicated doses (mg/kg per day). Red cell survival is expressed as the percent IgG-sensitized RBCs ($x \pm$ SEM) remaining in the circulation at each indicated time point. The clearances of unsensitized erythrocytes (unsensitized RBC control) and that of heat-damaged RBCs are shown. B represents the inhibition of the *in vitro* binding of IgG-sensitized RBCs by splenic macrophages isolated from guinea pigs treated with dopa-antagonists at the doses indicated on the abscissa. The percent splenic macrophages binding more than three IgG-sensitized RBCs ($x \pm$ SEM) compared to those of sham controls is shown. C indicates the percent inhibition ($x \pm$ SEM) of the mean fluorescence intensity for both classes of guinea pig macrophage Fcγ receptors, FcγR1,2 and FcγR2, by treatment with dopa-antagonists at the indicated doses (mg/kg per day).

erythrocytes by isolated splenic macrophages was 1 mg/kg for Clp, V, or SCH 23390. The administration of both V and SCH 23390 significantly impaired the *in vitro* binding of IgG-sensitized erythrocytes by splenic macrophages when compared to sham controls or animals treated with either SCH 23390 or V. Nevertheless, the administration of SCH 23390 (D1 antagonist) plus V (D2 antagonist) did not significantly impair the binding of IgG-sensitized erythrocytes when compared to that of the animals treated with Clp (a mixed D1/D2 dopa-antagonist).

Flow cytometry with specific monoclonal antibodies (Fig. 3C) demonstrated a significant inhibition of the splenic macrophage FcγR1,2 cell surface expression by 64 ± 3% for Clp at 25 mg/kg ($P < 0.001$), 54 ± 2% for V at 50 mg/kg ($P < 0.001$), 18 ± 1% for SCH 23390 at 25 mg/kg ($P < 0.01$), and 65 ± 3% for V at 50 mg/kg plus SCH 23390 at 25 mg/kg ($P < 0.001$). The expression of the other macrophage Fcγ receptor, FcγR2, was also inhibited by 56 ± 3% for Clp at 25 mg/kg ($P < 0.001$), 45 ± 2% for V at 50 mg/kg ($P < 0.001$), 7 ± 1% for SCH 23390 at 25 mg/kg ($P < 0.05$), and 56 ± 3% for V at 50 mg/kg plus SCH 23390 at 25 mg/kg ($P < 0.001$). These findings indicate that both D2 dopa-antagonists (V) and D1 dopa-antagonists (SCH 23390) inhibit the expression of macrophage Fcγ receptors. However, the inhibitory effect of D2 dopa-antagonists on macrophage Fcγ receptors expression is significantly more intense than that of D1 dopa-antagonists.

The above results taken together demonstrate that dopa-antagonists impair the expression of both guinea pig macrophage Fcγ receptors. This dopa-antagonist effect is mainly due to a D2 dopa-receptor-dependent action. Dopa-antagonists with a mixed D1/D2 dopa-receptor activity, like chlorpromazine, impair macrophage Fcγ receptors function and cell surface expression more effectively than selective D1 or D2 dopa-antagonists. We have not assessed the participation of other dopamine receptors classes on the observed dopaminergic regulation of macrophage Fcγ receptors expression.

Dopa-agonists decrease and, vice versa dopa-antagonists increase, the plasma levels of PRL (10, 11, 31–35). Therefore, the effects of dopaminergic agents on



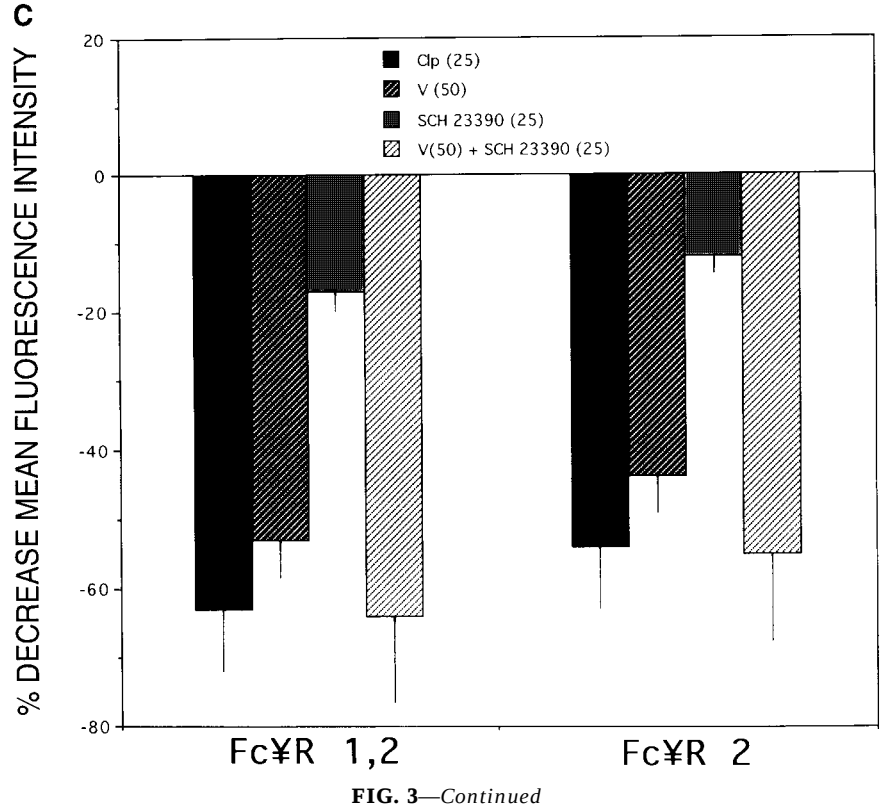


FIG. 3—*Continued*

macrophage Fcγ receptors expression may in part be due to altered PRL levels. Serum PRL was significantly decreased in animals treated with dopa-agonists and, vice versa, was elevated in animals treated with dopa-antagonists (data not shown). However, we did not find any correlation between PRL levels and the alteration of macrophage Fcγ receptors expression induced by treatment with dopaminergic drugs.

We report that treatment for 7 days with dopaminergic agents alters the Fcγ receptor mediated clearance of IgG-coated cells through an effect at the splenic macrophage Fcγ receptors expression. Dopa-agonists increased macrophage Fcγ receptors expression, whereas dopa-antagonists impaired macrophage Fcγ receptors expression. FcγR1,2 is more affected by dopaminergic drugs than the other Fcγ receptor class, FcγR2. This dopaminergic effect is mediated by both D1 and D2 dopamine receptors, with a more major role for D2 than for D1 receptors.

DISCUSSION

Macrophage Fcγ receptors have a critical role in host defense and the pathophysiology of immune mediated disorders. Impaired splenic macrophage Fcγ receptor clearance predisposes end-stage renal disease and cirrhotic patients to severe bacterial infections (1, 2). Defective splenic macrophage Fcγ receptors expression (35–40) and circulating anti-Fcγ receptors antibodies occur in several autoimmune diseases (41). The blockade or down-regulation of splenic macrophage Fcγ receptors expression is one of the mechanisms by which immune cytopenias improve (5–10, 42–45). The pharmacologic regulation of macrophage Fcγ receptors expression is a potential therapeutic intervention for immune mediated disorders. Dopaminergic drugs have important immunoregulatory functions (11–21, 28).

We examined one aspect of the pathologic process in autoimmune disorders, namely macrophage clearance of IgG-sensitized cells in the spleen, using an experimental model developed in the guinea pig, which has proved helpful in studying the effects of glucocorticoids and sex hormones in these conditions (3, 4, 8, 9). In doing so, we chose three dopa-agonists, bromocryptine, leuprolide, and pergolide, and six dopa-antagonists, chlorpromazine, metochlopramide, sulpiride, veralipride, alizapride, and cisapride. Bromocryptine is a dopa-agonist drug used in the treatment of movement disorders and to interrupt lactation by its inhibitory effects on prolactin secretion (25). Leuprolide (26) and pergolide (27) are synthetic dopa-agonists employed in the treatment of human conditions, mainly Parkinson's disease and prostate cancer. Chlorpromazine is a dopa-antagonist of the phenothiazine family that has been widely employed as a neuroleptic drug (28). M, S, V, A, and Cs are dopa-antagonists of the benzamide family. Metochlopramide has been widely used in the symptomatic treatment of digestive disorders (29). Sulpiride has been mainly prescribed for the treatment of minor psychiatric disorders (30). Veralipride has been employed in the symptomatic treatment of the postmenopausal syndrome with few side effects (31). Alizapride has been used in the symptomatic therapy of human digestive disorders (32). Cisapride is a new synthetic dopa-antagonist of the benzamide family, with selective D2 dopa receptor peripheral actions that are employed for the relief of nausea, vomiting, and gastroesophageal reflux (33).

Treatment for 7 days with dopaminergic agents altered the clearance of IgG-coated cells *in vivo,* the *in vitro* of IgG-sensitized RBCs by isolated splenic macrophages, and the cell surface expression of macrophage Fcγ receptors. Two Fcγ receptor classes, FcγR2 and FcγR1,2, have been identified in guinea pig macrophages (24). Our data suggest that these receptors are also expressed on essentially all splenic macrophages. Furthermore, when macrophages from the spleen are isolated, each of these Fcγ receptors participates in the binding of IgG-sensitized erythrocytes (9). Dopa-agonists increased the clearance of IgG-sensitized cells and enhanced the expression of both splenic macrophage Fcγ receptors, FcγR1,2 and FcγR2, while dopa-antagonists decreased splenic macrophage Fcγ receptors expression *in vivo* and *in vitro.* FcγR1,2 was significantly more sensitive to modulation by dopaminergic agents than FcγR2. Pergolide was the most effective dopa-agonist, while chlorpromazine was the most effective dopa-antagonist.

Chlorpromazine is a neuroleptic drug of the phenothiazine family, with high proautoimmune potential. It causes autoimmune disorders with a preponderant humoral component, namely a drug-related lupus (13, 14), immune thrombocytopenia, autoimmune hemolytic anemia, and frequent asymptomatic development of antinuclear antibodies, anticardiolipin antibodies, lupus anticoagulants, and increased serum levels of IgM (13, 15–18). Our findings of reduced macrophage Fcγ receptors function by dopa-antagonists treatment are in agreement with the suppressive effects of chlorpromazine on the cellular component of the immune response (19, 20, 46–50). Clp is a nonspecific dopamine antagonist which binds to all kinds of dopamine receptors (D1–D5), as well as to receptors for histamine and 5-hydroxytryptamine. Human lymphocytes and monocytes express dopamine receptors (51, 52). It is not known whether guinea pig splenic macrophages express dopamine receptors and, if so, of what specificity (D1 to D5). The multiple receptor mediated as well as nospecific effects of Clp make it particularly difficult to identify the molecular target(s) involved in Clp effects

on the immune system. It has been shown that the immunomodulatory effects of functional Clp analogues diminish when their specificity for D2 receptors augments (19, 50). Our data indicate that the dopaminergic alteration of macrophage Fcγ receptors function is a D1 and D2 dopamine receptor mediated effect, with a major D2 dopamine receptor participation.

Dopaminergic drugs alter endocrine function. Prolactin secretion is under dopaminergic control. Dopa-agonists decrease PRL levels, while dopa-antagonists induce hyperprolactinemia (53–57). Chronic hypo- or hyperprolactinemia is associated with humoral and cellular immune alterations that normalize with the restoration of normal PRL levels (11). Prolactin abnormalities have been described in a number of immunologic disorders, including SLE (11, 19), autoimmune uveitis, and thyroid disease (11, 12, 58, 59). These autoimmune disorders have been associated with an altered function of macrophage Fcγ receptors (35–40, 60–62), as well as with the presence of anti-Fcγ receptors autoantibodies (41, 63). Treatment with dopa-agonists was associated with hypoprolactinemia and, vice versa, dopa-antagonists induced hyperprolactinemia. Nevertheless, PRL levels did not correlate with macrophage Fcγ receptors alteration.

Our results indicate that: (1) Treatment with dopa-agonists bromocryptine, leuprolide, and pergolide enhances the clearance of IgG-sensitized cells while dopa-antagonists chlorpromazine, metochlopramide, sulpiride, veralipride, alizapride, and cisapride impair the clearance of IgG-sensitized cells by an effect on splenic macrophage Fcγ receptors expression, (2) Guinea pig macrophage receptor FcγR1,2 is more responsive to alteration by treatment with dopaminergic drugs than is the other macrophage Fcγ receptor, FcγR2, (3) The dopaminergic effects on macrophage Fcγ receptors expression are a mixed D1 and D2 dopamine-receptor-dependent action, with a major participation of D2 dopamine receptors and much less of D1 receptors. These effects can be achieved with doses of dopaminergic drugs close to those previously used for the treatment of human conditions. Changes of plasma prolactin levels induced by treatment with dopa-agonists or dopa-antagonists did not correlate with alterations of macrophage Fcγ receptors expression. Studies using nondopaminergic modulators of prolactin levels would help clarify the role of prolactin on macrophage Fcγ receptors expression. The potential implications of dopaminergic modulation of splenic macrophage Fcγ receptors expression will await clinical confirmation.

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