Clin. exp. Immunol. (1982) **47**, 103–109.

Beta-2-microglobulin-specific autoantibodies cause platelet aggregation and interfere with ADP-induced aggregation

A. FALUS, KATALIN MERÉTEY,* D. BAGDY,* MARIANNA DIÓSZEGI, UTE BÖHM, ÉVA CSÁK & S. BOZSÓKY *National Institute of Rheumatology and Physiotherapy, and * Institute for Drug Research, Budapest, Hungary*

(Accepted for publication 17 July 1981)

SUMMARY

The anti-platelet activity of beta-2-microglobulin (β_2m) specific autoantibodies isolated from sera of patients with autoimmune diseases was tested in direct and ADP-induced aggregation assays. It was established that human anti-β_2m autoantibodies and heterologous rabbit anti-β_2m antibodies evoke a dose-dependent aggregation of human platelets. Anti-β_2m autoantibodies also impaired ADP-induced platelet aggregation. Antibodies with anti-β_2m activity could be desorbed from the platelets and lymphocytes of a patient with systemic lupus erythematosus who was not thrombocytopenic. The possibility that such autoantibodies may alter platelet function is considered.

INTRODUCTION

The presence of human platelet antibodies and their possible deleterious effect on platelet functions and survival has been thoroughly investigated (Harrington *et al.*, 1951; Shulman, Marder & Weinrach, 1965; Dixon & Rosse, 1975). Antibodies responsible for clinically significant thrombopathies such as anti-HLA, anti-$P1^{A1}$, anti-ABO, drug-related and idiopathic thrombocytopenic purpura (ITP) related antibodies (Cimo *et al.*, 1977; Yankee, Grumet & Rogentine, 1969; Soulier, Patereau & Dronet, 1975; Aster, 1965) often also show lymphocytotoxic effects (Colman & Busch, 1977). Lymphocytotoxic autoantibodies specific for β_2m have recently been described in the sera from systemic lupus erythematosus (SLE) (Ooi *et al.*, 1977; Revillard, Vincent & Rivera, 1979) and rheumatoid arthritis (RA) (Falus, Merétey & Bozsóky, 1981) patients.

In this study we demonstrate that β_2m-specific autoantibodies isolated from the sera of RA and SLE patients can induce *in vitro* aggregation of platelets and modify their ADP-aggregation profiles.

MATERIALS AND METHODS

Patients. Serum was obtained from four patients with RA and extra-articular complications, two with SLE and one with juvenile RA. All patients with RA and SLE fulfilled the ARA criteria. At the time of investigation none of the patients suffered from thrombocytopenia (platelet count was over 100,000/mm^3). Platelet-rich plasma from 11 normal individuals was used in the experiments.

Anti-β_2m antibodies. IgG was prepared from normal human sera and anti-β_2m autoantibody-containing patient sera by ion-exchange chromatography on DEAE-cellulose (A-52). The IgG

Correspondence: Dr A. Falus, National Institute of Rheumatology and Physiotherapy, Division of Immunology, Budapest 114, POB 54, H-1525 Hungary.

0009-9104/82/0100-0103$02.00

preparations were reduced (10 mM dithiothreitol, 45 min, 20°C) and alkylated (25 mM iodoacetamide, 60 min, 4°C) according to Björk & Tanford (1971). Immunosorbent purification of anti-β_2m autoantibodies was performed on β_2m–Sepharose. Briefly, immunoelectrophoretically pure β_2m (kindly donated by Dr P. A. Peterson, Uppsala) was coupled to CNBr-activated Sepharose 4B (3·5 mg/ml wet gel) (Nardella & Mannik, 1978). Half a millilitre of the immunosorbent was mixed with 1·5 ml of anti-β_2m-containing IgG preparations (2–5 mg/ml) and incubated at 20°C for 2 hr and at 4°C for 16 hr. After exhaustive washing of the gel slurries, anti-β_2m antibodies were desorbed by addition of 1·5 ml of 0·1 M glycine–HCl buffer (pH 2·9) and subsequent incubation at room temperature for 10 min. The eluates were dialysed immediately against PBS (pH 7·4). Monospecific anti-human β_2m rabbit antibodies (IgG preparations from DAKO Immunoglobulins, Copenhagen, Denmark) were also used in some experiments.

Isolation of platelets and lymphocytes. Peripheral blood was withdrawn using citrate dextrose as anticoagulant. Platelet-rich plasma (PRP) was prepared by centrifugation at 200 ***g*** for 5 min in plastic tubes. Platelets were counted in a Coulter counter (Model Coulter Electronics) and adjusted to $1{\cdot}5–2 \times 10^5/mm^3$ with autologous plasma. Lymphocytes were isolated by Ficoll gradient centrifugation. The preparation contained 90–95% lymphocytes by morphology and 98% of the cells were viable by the trypan blue exclusion test.

Platelet aggregation. Half a millilitre of PRP and 0·1 ml of different dilutions of anti-β_2m, control IgG or physiological saline were stirred for 30 min at 37°C in polystyrene tubes. The direct increase in light transmission was followed in a Chronolog aggregometer and at the end of the incubation period, 3·3 μl of the suspension were diluted in 10 ml of diluent Isoton II and counted in a Coulter counter equipped with a 70-μl aperture. The degree of aggregation was estimated by calculating the percentage decrease in the number of unaggregated platelets compared with a control sample (Bull, Schneiderman & Brecher, 1965). In another series of experiments, 20 μl of a standard ADP (Calbiochem) solution at a final concentration of 5×10^{-5} M were added after a 30-min incubation of the PRP in the presence or absence of anti-β_2m autoantibodies and the aggregation curve was followed photometrically. The ADP response was examined by comparing the maximal increase in light transmission after 5 min in test and control suspensions. The inhibitions were expressed as percentages of the control values.

Elution and determination of anti-β_2m activity from platelets and lymphocytes. One hundred microlitres of PRP (10^7 platelets) and 0·2 ml of lymphocytes (10^7) from an SLE patient with circulating anti-β_2m antibody and a control subject were washed thoroughly three times with PBS (pH 7·4) and resuspended in 200 μl of glycine–HCl buffer containing 0·5 M glucose (pH 2·9). After a 5-min incubation at room temperature the mixtures were centrifuged (100 ***g***, 5 min) and the supernatants were dialysed immediately against PBS. The anti-β_2m activity of the desorbed material was determined by a modified Farr assay (Falus *et al.*, 1981). Briefly, 50 μl of the eluates were mixed with 50 μl of ^{125}I-β_2m (Pharmacia, sp. act. 93 μCi/μg) in the presence of 100 μl 0·1 M borate buffer (pH 8·1) containing 5% bovine serum albumin. After incubation for 16 hr at 4°C, 200 μl of saturated ammonium sulphate solution were added and the radioactivity of the washed precipitates was measured in a gamma counter (Autogamma, MOM, Budapest). The percentage of the total added radioactivity recovered as precipitable counts was calculated. The β_2m-binding activity of the sera from the tested subjects was also determined by the same method and compared to that of 140 μg and 1·4 μg of the commercial rabbit anti-human β_2m IgG preparation.

RESULTS

Continuous recording of light transmission of stirred platelet suspensions revealed a relatively fast aggregation induced by 2·8 mg/ml rabbit anti-human β_2m (Fig. 1a). Slower aggregation and an altered aggregation profile were observed after the addition of human autoantibody specific for β_2m, even at a concentration of 7·2 mg/ml (Fig. 1c). Very little or no increase in light transmission was found in the presence of lower concentrations of either rabbit or human anti-β_2m antibodies (Fig. 1b, d) and 10 mg/ml of control human or rabbit IgG (Fig. 1e, f).

A more sensitive assay was used in the majority of experiments which involved counting

Fig. 1. Platelet aggregation induced by anti-β_2m antibodies at 37°C. (a & b) Rabbit anti-human β_2-microglobulin IgG preparations, 2·8 and 1·4 mg/ml respectively; (c & d) human anti-β_2-microglobulin IgG preparations, 7·2 and 3·6 mg/ml respectively. (e & f) Curves obtained after adding control rabbit or human IgG preparations respectively (10 mg/ml).

unaggregated platelets after incubation with anti-β_2m antibodies. The effect on ADP-induced aggregation was also investigated in parallel. Serial dilutions of the monospecific rabbit anti-human β_2m (Table 1) caused a dose-dependent decrease in unaggregated platelet count and inhibited the ADP aggregation of the platelets. The dose–response curve of unaggregated platelet counts after incubation with a human anti-β_2m-containing IgG preparation is shown in Fig. 2.

Control preparations, containing 1 or 10 mg of normal human IgG, induced only a small decrease in platelet counts. The dose-dependent inhibition of ADP-induced aggregation by different concentrations of human anti-β_2m IgG preparations is demonstrated in Fig. 3. The ADP-induced platelet aggregation was greatly inhibited and there was some deaggregation with 500 μg of anti-β_2m IgG.

Data obtained with six human anti-β_2m-containing IgG preparations are shown in Table 2. The immunosorbent-purified anti-β_2m autoantibodies retained their platelet-aggregating activity and inhibitory effect on the ADP aggregation. The effects were compared to results obtained in parallel control experiments using the same platelet suspensions in the absence of anti-β_2m antibodies.

The *in vivo* binding of anti-β_2m antibodies to platelet and lymphocyte membranes was also investigated. Fractions eluted at pH 2·9 from the cells (10^7) were tested for anti-β_2m activity. In desorbed fractions from a patient with circulating anti-β_2m autoantibodies, anti-β_2m activity was

Table 1. Effect of monospecific rabbit anti-human β_2-microglobulin antibodies on the unaggregated platelet count of human platelets and on the aggregation induced by ADP

Rabbit anti-β_2m IgG* (μg)	Per cent decrease of unaggregated platelet count†	Per cent inhibition of ADP-induced aggregation‡
0·014	7·5±1·0	5
0·14	14·0±1·3	5
1·4	30·8±1·1	35·0
140·0	41·5±3·18	43·0
1,400·0	100§	n.d.

* 100 μl of IgG solutions were added to 500 μl of PRP suspensions and stirred at 37°C for 30 min.

† Compared to control values (mean±s.e.m., four experiments).

‡ Mean of two experiments.

§ No unaggregated platelets were detected.

¶ Not done because of complete aggregation of the platelets by 1,400 μg rabbit anti-human β_2m IgG.

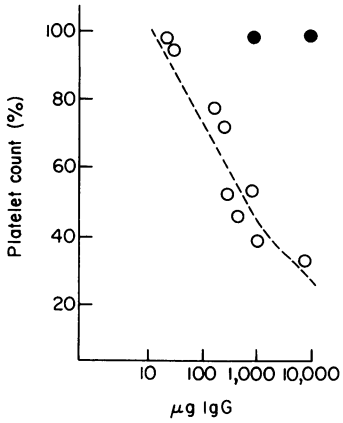

Fig. 2. Change of unaggregated platelet counts in platelet-rich plasma after 30 min of incubation with different amounts of human anti-β_2m IgG at 37°C. Normal human IgG (●– – – –●), anti-β_2m IgG (o– – – –o) (mean of three experiments).

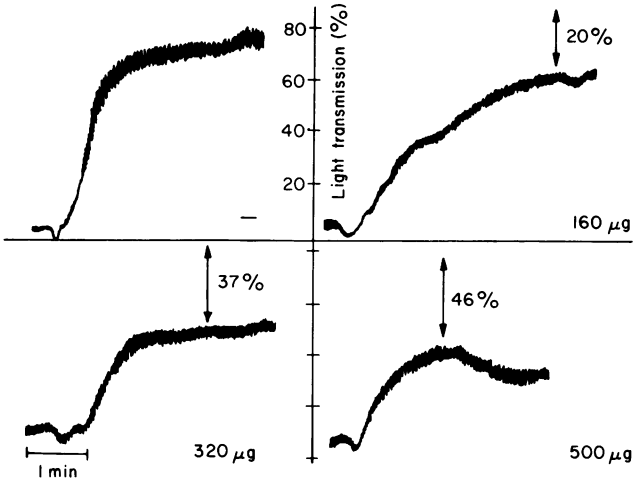

Fig. 3. Inhibition of ADP-induced (5×10^{-5} M) aggregation of human platelets by human anti-β_2m IgG. Amounts of added IgG and degree of inhibition are indicated. Inhibition is expressed as per cent decrease of ADP-induced aggregation of the same PRP suspension incubated without antibody.

detected (Table 3). The anti-β_2m activity of the desorbed antibodies was comparable to that of 1·4 μg of the rabbit anti-human β_2m IgG preparation.

Beta-2-microglobulin binding by the serum of a healthy control (3·1%) and patient K.L. with SLE (18·5%) were also determined (see Falus *et al.*, 1981). For comparison, 140 and 1·4 μg of rabbit anti-human β_2m antibodies showed binding percentages of 47·4 and 3·7% respectively.

DISCUSSION

The presence of HLA-A, B, C/β_2m complexes on the membrane of platelets has been well established (Holmud, Tülikainen & Penttinen, 1977) and Csáko, Suba & Wistar (1979) demonstrated the platelet-aggregating effect of rabbit anti-human β_2m antibodies. An anti-platelet activity of human anti-β_2m autoantibodies was indirectly indicated first by Wernet & Kunkel (1973) who showed that some SLE sera contained antibodies which reacted with a 12,000-dalton fraction

Table 2. Effects of six IgG preparations and immunosorbent-purified anti-β_2-microglobulin autoantibodies on the number of non-aggregated platelets and on ADP-induced aggregation

Patients* (sex)	Reduction in free platelet counts (%)†		Inhibition of ADP-induced aggregation (%)†	
	IgG‡ (200 μg)	Purified anti-β_2m§ (10 μg)	IgG‡ (200 μg)	Purified anti-β_2m§ (10 μg)
RA				
G.A. (F)	43	12	23	15
K.J. (F)	41	19·5	61	51
V.L. (F)	17	9	40	26
SLE				
D.V. (F)	18	7	35	27
K.L. (M)	45	29	24	17
JRA				
N.J. (F)	25	15	52	41

* Patients: F = female, M = male, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, JRA = juvenile rheumatoid arthritis.

† For conditions and evaluations, see Table 1 (Mean of two experiments).

‡ Reduced and alkylated IgG preparation from patient sera.

§ Purification of anti-β_2m autoantibodies was performed on Sepharose-β_2m as described under Materials and Methods.

Table 3. Anti-β_2-microglobulin antibodies desorbed from platelets and lymphocytes of an SLE patient and a control subject

Donor	Fractions eluted from:	β_2m binding* (%)
Healthy control	10^7 platelets	0·2
SLE patient	10^7 platelets	3·2
Healthy control	10^7 lymphocytes	0
SLE patient	10^7 lymphocytes	7·2

* Farr assay (see Materials and Methods), buffer control values are deducted (mean of two parallel determinations).

of human lymphocyte membranes, and that these antibodies could be removed by absorption with human platelets.

In this report we describe two effects of human anti-β_2m autoantibodies on platelets; their ability to aggregate platelets and to inhibit ADP-induced aggregation. Our results with immunosorbent-purified anti-β_2m autoantibodies strongly support the specificity of their interaction with platelets. Furthermore, preliminary observations (unpublished) on the aggregating effect of $F(ab')_2$ fragments of human anti-β_2m antibodies suggest that the binding is specific and not due to Fc interactions.

Because of the relatively low avidity and titre of anti-β_2m autoantibodies (Revillard *et al.*, 1979). a more sensitive system than photometric analysis was preferred in this study. When examined by phase-contrast microscopy (data not shown), autoantibodies to β_2m induced smaller platelet

aggregates than heterologous anti-β_2m antibodies, which explains the relative insensitivity of nephelometric evaluation in this type of study.

Interestingly, our SLE and RA patients with circulating autoantibodies to β_2m have not had thrombocytopenia in spite of the fact that their immunosorbent-purified anti-β_2m autoantibodies aggregated the platelets of healthy persons *in vitro*. Moreover, the finding of large amounts of anti-β_2m activity on the platelet membranes of an SLE patient with a normal platelet count shows that the *in vivo* effect of anti-β_2m autoantibodies can be subclinical. The recovery of anti-β_2m activity from both platelets and lymphocytes is of interest because of the association of both anti-platelet and lymphocytotoxic antibodies in some patients (Colman & Busch 1977).

We have shown that anti-β_2m antibodies inhibit ADP-induced aggregation of platelets. The appearance of a 'shape-change' in the first minutes after the addition of ADP indicates that β_2m-specific autoantibodies do not compete with the binding of ADP to the membranes. Kaplan & Nachman (1974) noted similar inhibitory effects of anti-platelet antibodies on the platelet aggregation induced by suboptimal concentrations of ADP, thrombin, collagen and epinephrine.

Our findings provide evidence for an *in vitro* interaction between anti-β_2m autoantibodies and platelets and point to the possibility of an *in vivo* role of anti-β_2m autoantibodies in processes leading to altered platelet functions. Apparent or latent platelet destruction may be induced by anti-platelet antibodies of other specificities (Levy-Toldano *et al.*, 1978), by immune complexes and by cell-mediated immune reactions to platelets (Morimoto, Abe & Homma, 1980). The contribution of anti-β_2m autoantibodies to the alteration of platelet function in different autoimmune processes and their relation to the activity of the disease is yet to be defined.

The authors wish to thank Dr Anna Erdei for preparing the reduced and alkylated IgG fractions. The help and criticism of Dr László Fésüs and Professor Sven-Erik Svehag in preparing the manuscript is also acknowledged. We thank Mrs Mária Vajda and Miss Elsebeth Orthmann for typing the manuscript.

This work was supported by Grant No. 6-07-0304-01-2/M from the Scientific Research Council, Ministry of Health, Hungary.

REFERENCES

Aster, R.H. (1965) Effect of anticoagulant and ABO incompatibility on recovery of transfused human platelets. *Blood*, **26**, 732.

Björk, I. & Tanford, C. (1971) Recovery of native conformation of rabbit immunoglobulin G upon recombination of separately renatured heavy and light chains at near-neutral pH. *Biochemistry*, **10**, 1289.

Bull, B.S., Schneiderman, M.A. & Brecher, G. (1965) Platelet counts with the Coulter counter. *Am. J. clin. Pathol.* **44**, 678.

Cimo, P.L., Pisciotta, A.V., Desai, R.G., Pino, J.L. & Aster, R.H. (1977) Detection of drug dependent antibodies by the ^{51}Cr platelet lysis test: documentation of immune thrombocytopenia induced by diphenylhydantoin, diazepam and sulfisoxasole. *Am. J. Hematol.* **2**, 65.

Colman, R.W. & Busch, G.J. (1977) Determination of anti-platelet antibody in sera cytotoxic for human lymphocytes. *Am. J. Hematol.* **2**, 211.

Csákó, G., Suba, E.A. & Wistar, R. (1979) Activation of human platelets by antibodies to thymocytes and β_2-microglobulin. I. Qualitative and quantitative aspects of the platelet aggregation induced by HATG and SAβ_2mG. *Clin. exp. Immunol.* **39**, 461.

Dixon, R.H. & Rosse, W.H. (1975) Platelet antibody in autoimmune thrombocytopenia. *Br. J. Haematol.* **31**, 129.

Falus, A., Merétey, K. & Bozsóky, S. (1981) Prevalence of anti-beta-2-microglobulin autoantibodies in sera of rheumatoid arthritis patients with extra-articular manifestations. *Ann. Rheum. Dis.* **40**, 409.

Harrington, W.J., Minnich, V., Hollingworth, J.W. & Moore, C.V. (1951) Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura. *J. Lab. clin. Med.* **38**, 1.

Holmud, G., Tülikainen, A. & Penttinen, K. (1977) The effect of HLA antibodies on platelet aggregation. *Scand. J. Immunol.* **6**, 157.

Kaplan, K.L. & Nachman, R.L. (1974) The effect of platelet membrane antibodies on aggregation and release. *Br. J. Haematol.* **28**, 551.

Levy-Toldano, S., Tobelem, G., Legrand, C., Bredoux, R., Degos, L., Nurden, A. & Caen, J.P. (1978) Acquired IgG antibody occurring in a thrombasthenic patient: its effect on human platelet function. *Blood*, **51**, 1065.

Morimoto, C., Abe, T. & Homma, M. (1980) Cell-mediated immunity to platelets in SLE patients with thrombocytopenia; two different types of lymphocyte stimulation. *Clin. Immunol. Immunopathol.* **15**, 1.

NARDELLA, F.A. & MANNIK, M. (1978) Nonimmunospecific protein interactions of IgG: studies of the binding of IgG to IgG immunoadsorbents. *J. Immunol.* **120,** 739.

OOI, B.S., OOI, Y.M., PESCE, A.J. & POLLAK, V.E. (1977) Antibodies to β_2-microglobulin in the sera of patients with systemic lupus erythematosus. *Immunology*, **33,** 535.

REVILLARD, J.P., VINCENT, C. & RIVERA, S. (1979) Anti-β_2-microglobulin lymphocytotoxic autoantibodies in systemic lupus erythematosus. *J. Immunol.* **122,** 614.

SHULMAN, N.R., MARDER, V.J. & WEINRACH, R.S. (1965) Similarities between known antiplatelet antibody and the factor responsible for thrombocytopenia in idiopathic purpura. Physiologic, serologic and isotopic studies. *Ann. N.Y. Acad. Sci.* **124,** 499.

SOULIER, J.P., PATEREAU, C. & DRONET, J. (1975) Platelet indirect radioactive Coombs' test. Its utilization for Pla_1 grouping. *Vox Sang.* **29,** 253.

WERNET, P. & KUNKEL, H.G. (1973) Demonstration of specific T lymphocyte membrane antigens associated with antibodies inhibiting the mixed leukocyte culture in man. *Transplant. Proc.* **5,** 1875.

YANKEE, R.A., GRUMET, F.C. & ROGENTINE, G.N. (1969) The selection of compatible platelet donors for refractory patients by lymphocyte HL-A typing. *N. Engl. J. Med.* **281,** 1208.