

High-affinity IgE receptor on eosinophils is involved in defence against parasites

Abdellilah Soussi Gounni*,
 Bouchaib Lamkhouloued*, Kenichi Ochial†,
 Yoichi Tanaka*, Emmanuel Delaporte‡,
 André Capron*, Jean-Pierre Kinet†
 & Monique Capron*§

* Centre d'Immunologie et de Biologie Parasitaire,
 Unite Mixte INSERM U167-CNRS624, Institut Pasteur,
 1 rue du Professeur A. Calmette, BP 245, 59019 Lille, France
 † Molecular Allergy and Immunology Section, National Institute of
 Allergy and Infectious Diseases, National Institutes of Health,
 Rockville, Maryland 20852, USA
 ‡ Service de Dermatologie A, Centre Hospitalier Régional
 Universitaire, 59000 Lille, France
 § To whom correspondence should be addressed

PARASITIC infections are often associated with eosinophilia and high levels of immunoglobulin E (IgE). This observation has led to speculation that eosinophils and IgE may act together in the immune response against parasites. In support of this hypothesis, IgE and eosinophils participate in cytotoxic reactions directed against *Schistosoma mansoni* larvae *in vitro*^{1,2}. Furthermore, epidemiological studies have shown an inverse correlation between levels of specific IgE and rates of infection with *Schistosoma*³⁻⁵. The low-affinity IgE receptor (FcεRII/CD23) was first incriminated in eosinophil activation^{6,7}. The fact that the high-affinity IgE receptor (FcεRI)^{8,9} is not only expressed on mast cells and basophils but also on Langerhans cells^{10,11} led us to investigate the presence of FcεRI on eosinophils. Here we show that FcεRI is expressed on eosinophils from hypereosinophilic patients, is involved in eosinophil degranulation, and participates in eosinophil-mediated cytotoxicity against *S. mansoni*. Our results indicate that FcεRI may play a major part in immune defence against parasites.

Highly purified eosinophils from hypereosinophilic patients were incubated with ¹²⁵I-labelled monomeric human IgE together with increasing concentrations of the anti-FcεRI α-chain monoclonal antibody 15-1 (ref. 10) or with a control antibody, and the cell-bound radioactivity counted. The monoclonal antibody 15-1, but not the isotypic control antibody, induces a

dose-dependent inhibition of monomeric IgE binding (Fig. 1). At the higher dose of 15-1 (50 µg ml⁻¹, inhibition is comparable to the inhibition seen with 1 mg ml⁻¹ of unlabelled human IgE. Under the same conditions, 1 mg ml⁻¹ of human IgG did not inhibit IgE binding, whereas 15-1 was not able to inhibit the binding of ¹²⁵I-labelled human IgG (data not shown). We then used flow cytometry to confirm the surface expression of FcεRI α-chain on the eosinophils. The FcεRI⁺ human basophil cell line KU812 served as a positive control. As expected, 90% of the KU812 cells stain positively with 15-1 (Fig. 2a) but not with control antibody. Under the same conditions, positive staining is detected on 13-73% of cells in purified eosinophil preparations (Fig. 2b, c). This percentage varies according to individual donors and is equivalent to binding of IgE (Fig. 2c). Immunostaining experiments with 15-1 were also performed on cyto-centrifuged KU812 cells or purified blood eosinophil preparations. Positive staining is again found on KU812 cells (Fig. 2d), as well as on some eosinophils (Fig. 2f). In contrast, no staining is detectable with the control antibody (Fig. 2e-g). Moreover, combined immunostaining of tissue eosinophils with 15-1 and an antibody against eosinophil cationic protein (ECP) shows that the 15-1-positive cells are eosinophils (Fig. 2h, i). Taken together, these data demonstrate that eosinophils from these patients can bind monomeric IgE through the FcεRI α-chain and that expression of FcεRI varies between individuals.

We next investigated whether transcripts for the FcεRI α-chain^{12,13}, which contains the binding site for IgE^{14,15}, could be detected by northern blotting in the control cell line KU812 or in the highly enriched eosinophil preparations. As expected, a 1.2-kilobase (kb) transcript is readily detected in KU812 cells (Fig. 3a). Eosinophils from all patients express transcripts of similar size, but with variable abundance according to the individual donor (Fig. 3a, lanes 2-5). No transcript is detected in preparations of lymphocytes or neutrophils (Fig. 3b, lanes 2-5), which may contaminate the eosinophil preparations. Furthermore, a similarly sized mRNA is also detected in butyrate-treated HL60 cells, which differentiate towards eosinophils^{16,17} (Fig. 3b, lanes 6 and 7), but not in untreated cells (Fig. 3a, lane 6; Fig. 3b, lane 8). This is remarkable not only because FcεRI α-chain transcripts are expressed in an eosinophil cell line, but also because this expression can be regulated.

FcεRI is a tetrameric structure composed of an IgE-binding α-chain, a β-chain and two disulphide-linked γ-chains^{8,9}. We tested for the presence of β- and γ-transcripts in our eosinophil preparations. As in basophils, the amount of β-chain mRNA is much less than for α-chain¹⁸, so the polymerase chain reaction

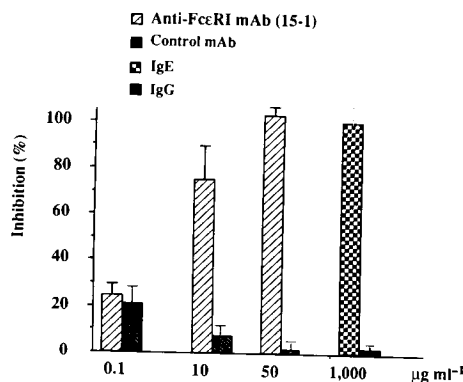


FIG. 1 Inhibition of ¹²⁵I-IgE binding to human eosinophils by anti-FcεRI α-chain monoclonal antibody (mAb) 15-1.

METHODS. Human eosinophils were purified from the venous blood of patients with eosinophilia, associated mainly with idiopathic hypereosinophilic syndrome (HES), skin diseases or lymphomas, by centrifugation on discontinuous 18-25% (w/v) metrizamide gradients (Nyegaard, Norway) as described²². The degree of purity of eosinophil populations, estimated after staining with Giemsa, was between 85 and 99%. Human IgE myeloma protein PS (gift from H. L. Spiegelberg) was radioiodinated by the chloramine-T technique⁷ to a specific activity of 1 × 10⁶ c.p.m. per µg. Purified human eosinophils (3.3 × 10⁶ cells per ml) were incubated with ¹²⁵I-labelled IgE myeloma protein (1 µg ml⁻¹) in a total volume of 60 µl for 90 min at 4 °C in the presence of various concentrations of anti-FcεRI α-chain mAb 15-1, or isotypic control mAb, or human IgE myeloma protein PS, or normal human IgG (Sigma). Cells were then centrifuged through sucrose gradients and cell-associated radiolabelled IgE was measured in a γ-scintillation counter. All results obtained with anti-FcεRI α-chain mAb 15-1 or control antibodies are presented as mean percentages of inhibition ± s.e.m. by comparison to those obtained with unlabelled IgE (n = 4 experiments). In two experiments, the average numbers of receptors per eosinophil were estimated to be 84,000 and 73,000 respectively.

(PCR) was used to detect β -chain mRNA. The amplified products obtained from eosinophils, KU812 cells and, remarkably, from butyrate-treated HL60, revealed the size bands predicted for Fc ϵ RI β - and γ -chains (Fig. 3c, d). The specificity of the fragments was confirmed by restriction enzyme analysis (data not shown) and Southern blotting, with internal oligonucleotides specific for each subunit (Fig. 3e, f). Thus, eosinophils and

differentiated HL-60 cells express α -, β - and γ -transcripts.

Effector functions of eosinophils appear to be primarily mediated by the release of granule proteins¹⁹. Moreover, IgE-dependent activation of eosinophils from patients with parasitic infections or with allergic diseases can lead to the selective release of eosinophil peroxidase (EPO) but not ECP^{20,21}. We therefore investigated whether crosslinking of Fc ϵ RI could induce EPO

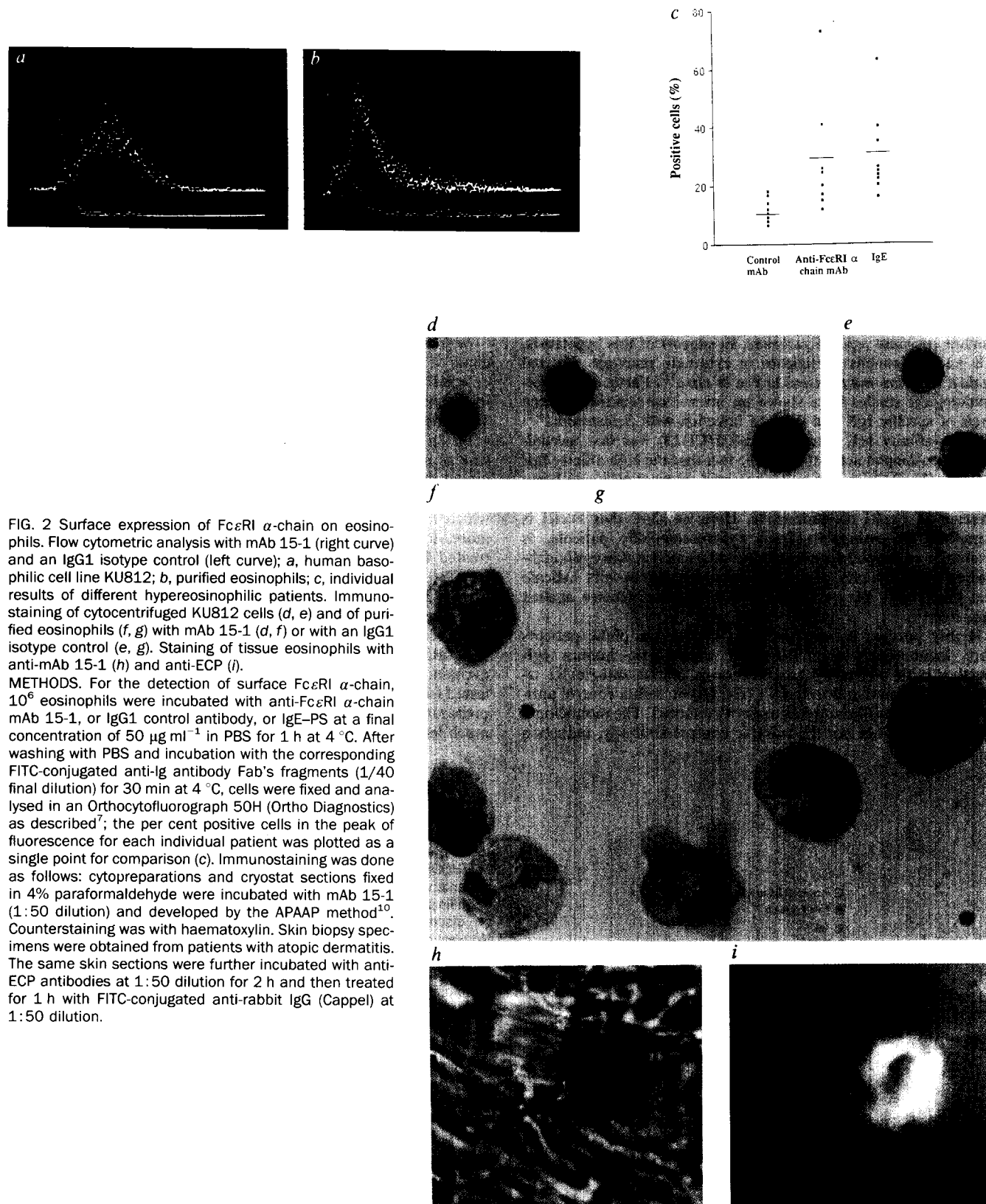
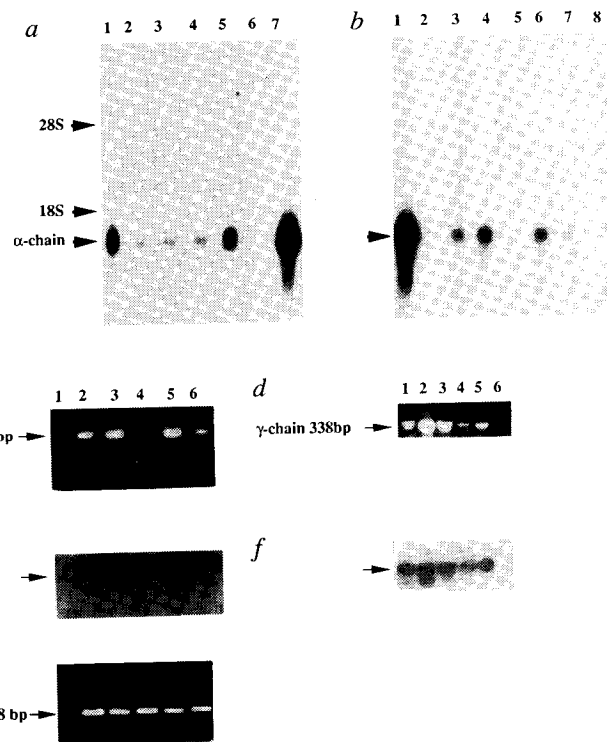


FIG. 2 Surface expression of Fc ϵ RI α -chain on eosinophils. Flow cytometric analysis with mAb 15-1 (right curve) and an IgG1 isotype control (left curve); a, human basophilic cell line KU812; b, purified eosinophils; c, individual results of different hypereosinophilic patients. Immunostaining of cytocentrifuged KU812 cells (d, e) and of purified eosinophils (f, g) with mAb 15-1 (d, f) or with an IgG1 isotype control (e, g). Staining of tissue eosinophils with anti-mAb 15-1 (h) and anti-ECP (i).

METHODS. For the detection of surface Fc ϵ RI α -chain, 10^6 eosinophils were incubated with anti-Fc ϵ RI α -chain mAb 15-1, or IgG1 control antibody, or IgE-PS at a final concentration of $50 \mu\text{g ml}^{-1}$ in PBS for 1 h at 4°C . After washing with PBS and incubation with the corresponding FITC-conjugated anti-Ig antibody Fab's fragments (1/40 final dilution) for 30 min at 4°C , cells were fixed and analysed in an Orthocytofluorograph 50H (Ortho Diagnostics) as described⁷; the per cent positive cells in the peak of fluorescence for each individual patient was plotted as a single point for comparison (c). Immunostaining was done as follows: cytopreparations and cryostat sections fixed in 4% paraformaldehyde were incubated with mAb 15-1 (1:50 dilution) and developed by the APAAP method¹⁰. Counterstaining was with haematoxylin. Skin biopsy specimens were obtained from patients with atopic dermatitis. The same skin sections were further incubated with anti-ECP antibodies at 1:50 dilution for 2 h and then treated for 1 h with FITC-conjugated anti-rabbit IgG (Cappel) at 1:50 dilution.

FIG. 3 Presence of FcεRI α-, β- and γ-chain transcripts in eosinophils. **a** and **b**, Northern blot hybridization of total RNA with a human FcεRI α-chain probe. **a**, Lanes: 1 and 7, human basophilic cell line KU812; 2–5, highly purified eosinophils from 4 different eosinophilic patients; 6, undifferentiated HL60 cells. **b**, Lanes: 1, KU812 cells; 2, purified lymphocytes from hyper-eosinophilic patient; 3 and 4, purified eosinophils, as in lanes 3 and 4 in **a**; 5, highly purified neutrophils from eosinophilic patient; 6 and 7, butyrate-induced HL60 cell line, at 7 and 3 days respectively; 8, undifferentiated HL60 cells. **c** and **e**, Reverse transcriptase (RT)-PCR and Southern blot analysis of FcεRI β-subunit. Lanes: 1, negative control (no cDNA); 2 and 6, purified eosinophils; 3, KU812; 4, undifferentiated HL60 cells; 5, differentiated HL60 cells. **d** and **f**, RT-PCR and Southern blot analysis of FcεRI γ-subunit. Lanes: 1 and 4, purified eosinophils; 2, differentiated HL60 cells; 3, undifferentiated HL60 cells; 5, KU812 cells; 6, negative control (no cDNA). **g**, RT-PCR with β-actin primers.

METHODS. Neutrophil preparations: patient neutrophils were obtained from the venous blood of patients with hypereosinophilia by centrifugation through discontinuous metrizamide gradients²³. Cell culture: a sub-line of the HL60 cell line (clone 15; gift from J. Tavernier (Roche Research, Gent, Belgium)) was maintained in RPMI 1640 medium supplemented with 10% FCS and 25 μg ml⁻¹ gentamicin. For induction, cells were incubated at 2 × 10⁵ per ml with 0.5 mM sodium butyrate for 7 days¹⁷. For northern blot analysis, 10 μg total RNA was used per lane⁹. The BamHI-XhoI fragment of the human FcεRI α-chain cDNA was used as the probe. RT-PCR: total RNAs from eosinophils, KU812 and HL60 cell line were reverse-transcribed and examined by PCR analysis using specific primers for β- and γ-chains of FcεRI (ref. 11). PCR conditions were 1 min at 94 °C, 2 min at 60 °C and 3 min at 72 °C for 30 cycles. PCR products were electrophoresed on agarose and visualized by ethidium staining. Southern blot analysis: Amplified products were blotted onto Hybond N membranes using standard methods. Oligonucleotide probes were labelled with T4 polynucleotide kinase and [³²P]ATP. Hybridization was carried out in the same conditions as before.



Oligonucleotides specific for sequences on either side of a splice junction within β and γ genes were used in the PCR reaction to preclude amplification of possible contaminating genomic DNA. The FcεRI β- and γ-chain primers have been described¹¹.

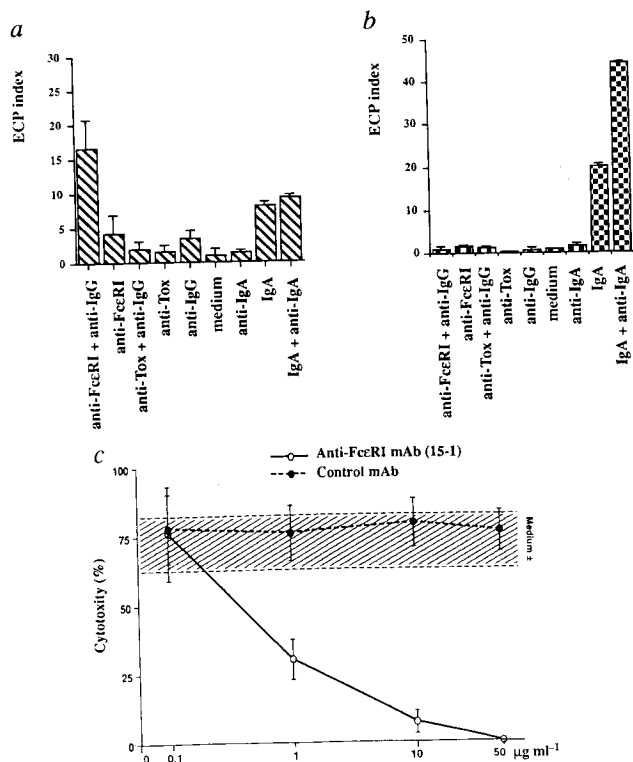
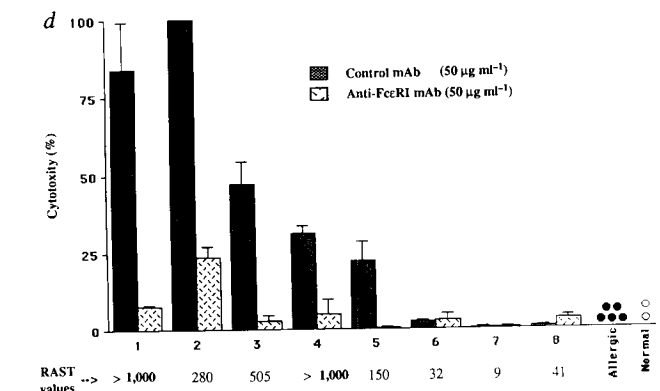


FIG. 4 **a** and **b**, Release of EPO and ECP after stimulation of eosinophils with anti-FcεRI mAb. **c** and **d**, Inhibition of eosinophil-mediated IgE-dependent cytotoxicity against schistosome targets. For cell activation, highly purified eosinophils (>90%) were incubated with anti-FcεRI α-chain mAb 15-1, irrelevant mAb of the same isotype, or secretory IgA (positive control) at a final concentration of 10 μg ml⁻¹.



at 4 °C. Then cells were challenged with anti-Ig antibody Fab/2 fragments and incubated for 2 h at 37 °C in 5% CO₂. As negative controls, cells were incubated with each reagent alone. EPO and ECP release was evaluated in the same supernatants by a chemiluminescence assay²⁰ and a double-antibody radioimmunoassay method (Pharmacia Diagnostics) respectively. Results are expressed as the index of EPO and ECP release as described²¹. For cytotoxicity assay, eosinophils were preincubated with various concentrations of anti-FcεRI mAb 15-1 or isotype control for 30 min at room temperature before addition to *S. mansoni* targets⁹, in the presence of one IgE containing reference immune serum (at 1:32 final dilution; total volume, 200 μl). Results were compared with those obtained with targets incubated without mAb (medium) in the presence of effector cells and immune serum. The cytotoxicity values obtained with eosinophils from 4 different eosinophilic donors were expressed as mean % cytotoxicity ± s.e.m. **d**, Individual results of sera (final dilution 1:32) from 8 different *S. mansoni*-infected patients (patients 1–8), containing varying amounts of specific anti-*S. mansoni* IgE antibodies, were evaluated by a radioallergosorbent test against a soluble *S. mansoni* worm extract. IgE values are expressed as the index of bound radioactivity versus normal serum (RAST values). Anti-FcεRI (15-1) or control isotype mAb were used as in **c** (mean cytotoxicity values of 3 experiments ± s.e.m.). Sera from 5 allergic individuals containing between 420 and 4,313 IU ml⁻¹ total IgE were used as specificity controls. Normal human sera were used as negative controls.

release from eosinophils (Fig. 4a). Increased levels of EPO (index of release >15) are detected after stimulation of purified eosinophils by crosslinking antibody 15-1 but not an unrelated isotype control antibody, or other control antibodies. In contrast, no significant amounts of ECP were detected in the same supernatants in which EPO was measured (Fig. 4b). However, elevated levels of both EPO and ECP are obtained in supernatants of the same eosinophil preparations after crosslinking of secretory IgA (Fig. 4a, b). These data indicate that FcεRI is involved in the differential release of EPO versus ECP, as was shown previously after IgE-dependent activation^{20,21}.

Finally, we analysed whether FcεRI is involved in the cytotoxicity of eosinophils against parasites. Purified eosinophils, schistosomula targets, and IgE antibody-containing immune sera were incubated in the presence of antibody 15-1 or an isotype control antibody. A dose-dependent inhibition of cytotoxicity is

observed with 15-1 but not with control antibody in the presence of one immune serum used as positive control (Fig. 4c). Also, when more sera from *S. mansoni*-infected patients were used, not only were cytotoxicity levels significantly correlated with specific anti-*S. mansoni* IgE levels (Spearman's correlation test, $P < 0.05$), but they were also significantly inhibited by 15-1 at the optimum concentration (Fig. 4d). None of the five control isotype sera from allergic patients was able to induce target cell lysis. Anti-CD23 monoclonal antibody of the same isotype (antibody 135)⁷ used at $50 \mu\text{g ml}^{-1}$ did not significantly inhibit cytotoxicity (data not shown). These results indicate that FcεRI can mediate eosinophil-dependent cytotoxicity against schistosomula and provide the evidence that, in addition to its role in mediating allergic responses^{8,7}, FcεRI may participate in a physiological protective immune response against metazoan parasites. □

Received 29 July; accepted 16 November 1993.

1. Capron, A. et al. *Immun. Today* **7**, 15–18 (1986).
2. Capron, A., Dessaint, J. P., Capron, M., Ouma, J. H. & Butterworth, A. E. *Science* **238**, 1065–1072 (1987).
3. Hagan, P., Blumenthal, U. J., Dunne, D., Simpson, A. J. & Wilkins, H. A. *Nature* **349**, 243–245 (1991).
4. Rihet, P., Demeure, C. E., Bourgeois, A., Prata, A. & Dessein, A. J. *Eur. J. Immun.* **21**, 2679–2686 (1991).
5. Dunne, D. W. et al. *Eur. J. Immun.* **22**, 1483–1486 (1992).
6. Capron, M., Grangette, C., Torpier, G. & Capron, A. *Chem. Immun.* **47**, 128–178 (1989).
7. Capron, M. et al. *Eur. J. Immun.* **21**, 2423–2429 (1991).
8. Kinet, J.-P. in *Inflammation: Basic Principles and Clinical Correlates* 701–708 (Raven, New York, 1992).
9. Ravetch, J. V. & Kinet, J. P. *Rev. Immun.* **9**, 457–479 (1991).
10. Wang, B. et al. *J. exp. Med.* **175**, 1353–1358 (1992).
11. Bieber, T. et al. *J. exp. Med.* **175**, 1285–1291 (1992).
12. Kochan, J., Pettine, L. F., Hakimi, J., Kisch, K. & Kinet, J. P. *Nucleic Acids Res.* **16**, 3584–3594 (1988).

13. Shimizu, A. et al. *Proc. natn. Acad. Sci. U.S.A.* **85**, 1907–1911 (1988).
14. Blank, U., Ra, C. & Kinet, J. P. *J. biol. Chem.* **266**, 2639–2646 (1991).
15. Hakimi, J. et al. *J. biol. Chem.* **265**, 22079–22081 (1990).
16. Fischkoff, S. A. et al. *J. exp. Med.* **160**, 179–196 (1984).
17. Plaetinck, G. et al. *J. exp. Med.* **172**, 683–691 (1990).
18. Kuster, H., Zhang, L., Brini, A. T., MacGlashan, D. W. J. & Kinet, J. P. *J. biol. Chem.* **267**, 12782–12787 (1992).
19. Gleich, G. J. & Adolphson, C. R. *Adv. Immun.* **39**, 177–253 (1986).
20. Khalife, J. et al. *J. Immun.* **137**, 1659–1664 (1986).
21. Tomassini, M. et al. *J. Allerg. clin. Immun.* **88**, 365–375 (1991).
22. Capron, M. et al. *J. exp. Med.* **164**, 72–89 (1986).
23. Truong, M. J. et al. *J. exp. Med.* **177**, 243–248 (1993).

ACKNOWLEDGEMENTS. We thank J. P. Dessaint, M. Joseph and M. H. Jouvin for advice; A. Scharenberg for critically reading the manuscript; J. P. Papin, S. Loiseau, J. L. Neyrink and J. P. Kusnier for help with experimental procedures; K. Nilsson for the KU812 cell line; and J. Pestel and P. Desreumaux for human sera. A. S. G. is supported by Fondation Marcel Merieux, Lyon, France.

Diadenosine phosphates and the physiological control of blood pressure

Hartmut Schlüter, Elmar Offers, Gitta Brüggemann, Marcus van der Giet, Martin Tepel, Eckhard Nordhoff*, Michael Karas*, Claus Spieker, Herbert Witzel† & Walter Zidek

Medizinische Poliklinik, *Institut für Medizinische Physik, † Institut für Biochemie der Universität Münster, Albert Schweitzer Strasse 33, Münster 48129, Germany

OUR understanding of the regulation of vascular tone has been extended since the identification of vasoactive agents such as the atrial natriuretic peptides¹, endothelial-derived relaxing factor² and endothelin³. Unidentified vasopressor agents have been found in platelets⁴. Here we isolate these vasopressors and identify them as diadenosine pentaphosphate (AP₅A) and diadenosine hexaphosphate (AP₆A) by chromatography, mass spectrometry, ultraviolet spectroscopy and enzymatic cleavage. In the vasculature of isolated perfused rat kidney, both diadenosine phosphates were active at a concentration of 10^{-9} M; in aortic rings, contractions were elicited at 10^{-8} M. Intra-aortic injection in the rat caused a prolonged increase in blood pressure. We conclude that AP₅A and AP₆A may play a part in local vasoregulation and possibly in the regulation of blood pressure.

The vasopressors were purified from platelet concentrates in the following steps (Fig. 1): (1) deproteinization with perchloric acid; (2) binding to a preparative C18 column using triethylammonium acetate as a cationic ion-pair reagent; (3) displacement chromatography on a second C18 column (Fig. 1a); (4)

displacement chromatography on an anion-exchange column (Fig. 1b); and (5) gradient elution with triethylammonium acetate as a cationic ion-pair reagent on a third C18 column (Fig. 1c, d). After each chromatographic step, vasopressor activity of the fractions was assayed using an isolated perfused rat kidney (Fig. 1 and Fig. 2a, b, g). After the last purification step, a single homogeneous peak was obtained (Fig. 1e, f).

The active substances were identified as diadenosine pentaphosphate (AP₅A) and diadenosine hexaphosphate (AP₆A) by the following findings (Fig. 3): (1) matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) revealed relative molecular masses of 916 (Fig. 3a) and 996 (Fig. 3b); (2) after addition of Na⁺, MALDI mass spectrometry showed that there were five and six negative charges in AP₅A and AP₆A, respectively (Fig. 3c, d); (3) ultraviolet spectroscopy gave a spectrum characteristic of adenine (Fig. 3e, f); (4) the retention times of the active fractions in step 5 were identical to those of commercially available AP₅A and AP₆A; and (5) MALDI-MS of commercially available AP₅A and AP₆A with and without a Na⁺ salt gave the same pattern as the fractions in step 5. The structures of AP₅A and AP₆A were evidenced by the following results (Fig. 3c, d, g and h): (1) MALDI analysis revealed five and six negative charges, respectively; however, isomers of AP₅A and AP₆A with one terminal phosphate group in the 3' position would have six and seven negative charges as one terminal phosphate group would introduce two negative charges into the molecule; (2) both compounds were resistant to alkaline phosphatase; (3) snake venom phosphodiesterase (5' nucleotidase) yielded AMP plus AP₄ and AP₅, respectively, whereas calf spleen phosphodiesterase (3' nucleotidase), which is specific for 3' linkages, was ineffective. The molar ratio of ATP to AP₅A and AP₆A in the platelet extracts was estimated to be in the range of 50:1 and 100:1. ATP content was estimated chromatographically⁵. When platelets were aggregated with 1 U ml^{-1} thrombin (Sigma), AP₅A and AP₆A were isolated from the supernatant by the purification procedure already described.