also a simple model for investigation of the requirements for NK maturation *in vivo*.

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1. Herberman, R. B. (ed.) *NK Cells and Other Natural Effector Cells* (Academic, New York, 1982).
2. Riccardi, C., Puccetti, P., Santoni, A. & Herberman, R. B. *J. natn. Cancer. Inst.* **63,** 1041–1047 (1979).
3. Karre, D., Klein, G. O., Kiessling, R. & Roder, J. C. *Nature* **284,** 624–626 (1980).
4. Hanna, N. & Burton, R. C. *J. Immun.* **127,** 1754–1758 (1981).
5. Warner, J. F. & Dennert, G. *Nature* **300,** 31–34 (1980).
6. Herberman, R. B. in *Biological Mediators of Behaviour and Disease: Neoplasia* (ed. Levy, S.) (Elsevier, Amsterdam, in the press).
7. Bindoni, M., Belluardo, N., Licciardello, S., Marchese, A. E. & Cicirata, F. *Expl Neurol.* **67,** 433–437 (1980).
8. Bindoni, M., Belluardo, N., Licciardello, S., Marchese, A. E. & Cicirata, F. *Neuroendocrinology* **30,** 88–93 (1980).
9. Bindoni, M., Belluardo, N., Marchese, A. E., Mudo', G. & Laguidara, A. *Neuroendocr. Lett.* **3,** 347–363 (1981).
10. Timonen, T., Saksela, E., Ranki, A. & Hayry, P. *Cell Immun.* **48,** 133–140 (1979).
11. Kumagai, K., Itoh, K., Suzuki, R., Hinuma, S. & Saitoh, F. *J. Immun.* **129,** 388–395d (1982).
12. Lehmann, A. *Atlas Stereotaxique du Cerveau de la Souris* (Centre National Recherche Scientifique, Paris, 1974).
13. Santoni, A., Riccardi, C., Sorci, V. & Herberman, R. B. *J. Immun.* **126,** 2329–2332 (1980).
14. Giovarelli, M., Landolfo, S., Whitmore, A. C. & Forni, G. *Eur. J. Cancer* **18,** 307–315 (1982).
15. Landolfo, S., Giovarelli, M., Viano, I. & Forni, G. *Immunogenetics* **9,** 245–253 (1979).
16. Julius, M., Simpson, E. & Herzenberg, L. W. *Eur. J. Immun.* **3,** 465–469 (1973).
17. Santoni, A., Herberman, R. B., Piccoli, M. & Ortaldo, J. *Fedn Proc.* **42,** 1380 (1983).
18. Trinchieri, G. & Santoli, D. *J. exp. Med.* **143,** 569–682 (1978).
19. Riccardi, C., Vose, B. M. & Herberman, R. B. in *NK Cells and Other Natural Effector cells* (ed. Herberman, R. B.) 909–915 (Academic, New York, 1982).
20. Djeu, J. Y., Heinbaugh, J. A., Holden, H. T. & Herberman, R. B. *J. Immun.* **122,** 175–181 (1979).
21. Kuribayashi, K., Gillis, S., Kern, D. E. & Henney, C. S. *J. Immun.* **126,** 2321–2326 (1981).
22. Saxena, Q. B., Saxena, R. K. & Adler, W. H. *Int. Archs. Allergy appl. Immun.* **67,** 169–174 (1982).
23. Talwor, G. P. *et al. Recent Prog. Horm. Res.* **31,** 141–174 (1975).
24. Fabris, N., Pierpaoli, W. & Sorkin, E. *Clin. exp. Immun.* **9,** 227–240 (1971).
25. Mathews, P. M., Froelich, C. J., Sibbit, W. L. & Bankhurst, A. D. *J. Immun.* **130,** 1658–1662 (1983).
26. Solomon, G. F. & Amkkraut, A. A. *A. Rev. Microbiol.* **35,** 155–184 (1981).

Antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes with secretory IgA

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Secretory antibodies of the IgA class (sIgA) are thought to have an important role in the defence against bacteria at mucosal surfaces—the level at which the infectious agents first come into contact with the host. However, the mechanism by which sIgA exert their antibacterial activity is still a matter of debate. After the recent discovery of receptors for the Fc portion of IgA (RFcα) on lymphocytes, monocytes and granulocytes of human, rabbit, guinea pig and mouse origin[1–6], it has been hypothesized that IgA also mediate antibody-dependent cellular cytotoxicity (ADCC). Indeed, ADCC mediated by human leukocytes against bacteria has been demonstrated in the presence of human circulating IgA[7–9]. As RFcα have also been shown to bind sIgA[2], we decided to investigate whether sIgA could mediate antibacterial ADCC when bound to lymphocytes from the murine gut-associated lymphoid tissues (GALT) which first interact with the invading bacteria. By using *Shigella* X16 (a hybrid strain between the enteric pathogen *Shigella flexneri* and *Escherichia coli*)[10] as target in an *in vitro* assay that measures cell-mediated antibacterial responses[11], we found that murine lymphocytes from GALT but not from other tissues are able to exert natural antibacterial activity against *Shigella* X16, and that sIgA significantly and specifically increase the natural antibacterial activity of GALT lymphocytes from mice and induce antibacterial activity in cells from the spleen, but not from the thymus or popliteal lymph nodes. Thus, we now propose a new role for sIgA in protecting the host against infectious agents at the mucosal level.

The intestinal secretions collected from chronically isolated rabbit ileal (Thiry-Vella) loops injected with *Shigella* X16 were used to obtain purified sIgA (<1.3 ng ml^{-1} of IgG in preparations with 0.6 mg ml^{-1} of sIgA)[12]. These antibodies were then incubated for 2 h at 37 °C with murine cells from different anatomical sites and *Shigella* X16. At the end of the incubation period, the pellets were vigorously resuspended (no bacteria adherent to leukocytes were evident by microscopic examination after this treatment) and appropriately diluted aliquots were plated on agar to assess colony-forming units (CFU)[11]. Table 1 shows two representative experiments performed with different effector-to-target ratios and an optimal sub-agglutinating antibody dilution (1/500), as determined by the antibody titration reported in Table 2. The data in Tables 1 and 2 show that lymphocytes from the epithelium and the lamina propria of the small intestine, from Peyer's patches and from mesenteric lymph nodes, all exert natural antibacterial activity. This confirms and extends recent observations obtained using *Salmonella typhimurium* in this *in vitro* assay[11]. Interestingly, the spontaneous antibacterial activity against *Shigella* X16 was peculiar to GALT lymphocytes, as cells from thymus, spleen or popliteal lymph nodes did not affect the bacterial growth by themselves. When purified anti-*Shigella* X16 sIgA were added to the *in vitro* assay, a significant increase in the antibacterial activity of GALT and spleen leukocytes was observed. The lack of effect of sIgA on cells from thymus and popliteal lymph nodes (Tables 1, 2), the absence of any increase in the anti-*Shigella* X16 activity with crude fluid from ileal loops of normal rabbits (data not shown), and the results of cross-reactivity experiments with *Salmonella tel aviv* (Table 3) reasonably rule out nonspecific effects in the system used. Moreover, the extremely low contamination with IgG of our preparation of sIgA[12] (the ratio IgG : sIgA was less than 2.1×10^{-6}) makes it highly unlikely that IgG-dependent activity has a role in our system, particularly with antibody dilutions as high as 1/500. Finally, direct evidence that sIgA and not contaminating antibodies of other classes determined the activity against *Shigella* X16 was obtained using sIgA purified on affinity columns. In fact, sIgA recovered from the column still showed antibacterial activity when bound to lymphocytes from normal mice (expt 3, Table 1). Further, it must be stressed that lymphocytes from Peyer's patches and mesenteric lymph nodes cannot exert IgG-mediated ADCC against bacterial and non-bacterial targets[11,13]. Thus, the sIgA-dependent antibacterial ADCC observed *in vitro* suggests a specialist function for the cells from these organs.

In view of the fact that only an incomplete reduction of bacterial viability was obtained, the ratios between effector cells and bacteria used in this *in vitro* assay (from 25 : 1 to 200 : 1) might seem too high, raising criticism as to their relevance to an *in vivo* situation. However, it must be considered that the *in vitro* doubling time of *Shigella* X16 is 20 min and that the effector : target ratio thus rapidly decreases during the 2 h assay. Furthermore, it is possible that only a minor fraction of the total population used would have antibacterial activity, as shown in many other assays measuring cell-mediated activities[14], and this would bring the actual effector : target ratios to much lower values. Finally, it has been reported that 90–95% of patients having Gram-negative bacterial sepsis have bacteraemias of $<1 \times 10^2$ bacteria per ml of peripheral blood[15]; thus, the *in vitro* system seems to resemble the *in vivo* pathological condition.

Previous studies[16,17] have shown that the population of cells obtained from the intestinal mucosa after Percoll fractionation comprises essentially lymphocytes. Thus, at least at this level, the effector cell of both natural and sIgA-dependent antibac-

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Table 1 Natural and sIgA-mediated antibacterial activity of intestinal and peripheral leukocytes against *Shigella* X16

Cell source	sIgA	Antibacterial index 25*	50	100	200	Statistical analysis
Expt 1 Spleen	—	—	−2	−7	−5	—
	1/500†	—	15	22	34	‡
Gut epithelium	—	—	13	22	36	—
	1/500	—	25	34	41	(3.5)§
Gut lamina propria	—	—	22	29	34	—
	1/500	—	40	44	45	(7.4)§
Peyer's patches	—	22	25	28	—	—
	1/500	31	41	45	—	(6.5)§
Expt 2 Spleen	—	—	0	−6	−2	—
	1/500	—	23	27	41	‡
Mesenteric lymph nodes	—	—	19	20	26	—
	1/500	—	33	38	42	(12.5)§
Popliteal lymph nodes	—	—	−4	−11	−9	—
	1/500	—	−6	−6	−4	NS
Peyer's patches	—	8	6	14	—	
	1/500	15	20	25	—	(8.4)§
Thymus	—	—	1	2	6	—
	1/500	—	3	6	6	NS
Expt 3 Spleen	—	—	−3	−3	−5	—
	1/500‖	—	6	16	22	‡
	1/100‖	—	22	34	36	‡
Peyer's patches	—	13	20	27	—	
	1/500‖	15	26	31	—	NS
	1/100‖	28	35	38	—	(3.4)§

Single cell suspensions were obtained from spleen, Peyer's patches and lymph nodes of 8-week-old C3H/HeN male mice by gentle teasing. Leukocytes from gut epithelium and lamina propria were obtained as described previously[16,17] and purified on Percoll gradients[17]. Antibacterial activity[7–9,11] was assessed as follows: 10^4 bacteria were placed 15-ml conical tubes together with either diluted anti-*Shigella* X16 sIgA antibodies or medium (RPMI 1640 plus 10% fetal bovine serum) and centrifuged at 1,300*g* for 10 min at 4 °C. Lymphocytes were then added and the tubes were again centrifuged at 500*g* for 5 min at 4 °C. To maintain the optimal proportion of reactants, the final volume of the mixture was 0.3 ml. The experimental and control tubes (which contained bacteria but no cells) were then incubated at 37 °C for 2 h, after which the pellets were vigorously resuspended, diluted and plated on Petri dishes with agar–tryptose. CFU were counted after overnight incubation. Duplicate tubes were set up for each experimental group and two Petri dishes were prepared for each tube. The percentage of antibacterial activity was expressed as an antibacterial index = 100 − [100 × (CFU of experimental tubes)/(CFU of control tubes without leukocytes)]. sIgA alone had no effect on bacterial survival. Antibacterial activity was analysed statistically by parallel line assay[21] after logarithmic transformation of the variables. Potency of experimental groups with sIgA versus no sIgA was estimated as the ratio between the number of cells of test and control groups giving equal antibacterial indices. Expts 1 and 2 are representative of 11 performed. The variability of the experimental system was low: for example, in 11 experiments the mean antibacterial indices ± s.e. for natural and antibody-mediated activity were, respectively, −7.5 ± 2.9 and 32.6 ± 2.1 for splenocytes at the 200:1 ratio, and 27.8 ± 3.0 and 43.3 ± 4.1 for leukocytes from Peyer's patches at the 100:1 ratio. Expt 3 was performed using sIgA purified in affinity chromatography columns containing anti-IgA antibodies (Miles, Elkhart) conjugated to CNBr-activated Sepharose 4B (Pharmacia).

* Effector:target bacteria (E:T) ratio.

† Antibody dilution.

‡ Control and test groups have significantly different slopes ($P \leq 0.05$).

§ Control and test groups have parallel slopes. Potency (in parentheses) is significant ($P \leq 0.05$). NS, not significant.

‖ Purified by affinity chromatography.

terial activity does not seem to be a macrophage (that is, a cell with a well established role in antibacterial reactions). Furthermore, preliminary experiments performed with cells from Peyer's patches indicated that the effector cell at this level is Thy 1.2^-, partially asialo-GM_1^+ and neither adherent nor phagocytic (data not shown). Thus, this cell seems to be phenotypically more similar to a null or K cell[14] than a macrophage.

Table 2 Titration of sIgA antibodies against *Shigella* X16

Cell source	Antibacterial index for sIgA diluted: No IgA	1:250	1:500	1:1,000	1:2,000	1:4,000
—	0	4	−1	3	3	5
Spleen*	5	14‡	37‡	30‡	14‡	4
Gut epithelium*	34	42	53‡	46‡	36	33
Peyer's patches†	27	32	43‡	40‡	36‡	27
Mesenteric lymph nodes*	30	36‡	46‡	42‡	36‡	30
Popliteal lymph nodes*	2	2	2	2	4	3
Thymus*	0	2	2	5	3	2

Experimental conditions as in Table 1.

* E:T ratio, 200:1; † E:T ratio, 100:1.

‡ $P \leq 0.05$ versus no sIgA as assessed by analysis of variance.

Table 3 Specific effect of sIgA against *Shigella* X16

Cell source	sIgA anti-*Shigella* X16*	IgA anti-*S. tel aviv**	Antibacterial index against: *Shigella* X16	*S. tel aviv*
Spleen†	—	—	−2	30
	—	+	4	59§
	+	—	40§	26
Peyer's patches‡	—	—	32	−28
	—	+	27	20§
	+	—	46§	−16

Experimental conditions were as described in Table 1. IgA antibodies against *S. tel aviv* (from our own bacterial collection) were obtained from ascitic fluid of BALB/c mice injected with MOPC 384 plasmacytoma[22].

* Dilution 1:500; † E:T ratio 200:1; ‡ E:T ratio 100:1.

§ Significant difference ($P \leq 0.05$) compared with corresponding control without antibodies, assessed as in Table 2.

Indeed, an increasing number of reports suggest that, besides macrophage-dependent activities, cell-mediated responses such as natural killer (NK) activity and ADCC, at first defined as effector mechanisms against neoplastic cells, might also have a role against viruses, fungi, bacteria and protozoa[18]. As many infectious agents contact the host at the mucosal level, it might be expected that these immunosurveillance mechanisms should be especially active at these anatomical sites. It is striking that large granular lymphocytes (LGL)—the main effector cells of NK activity and ADCC against tumour cells in many species[19,20]—are present in high numbers in the intestinal epithelium and lamina propria, and are still able to exert NK activity against tumour cells at this level[16,17]. As NK and ADCC seem to be different functions of the same lymphocyte, that is, the LGL[18,19], it might be suggested that the gut LGL also have a role in the antibacterial sIgA-dependent ADCC.

Thus, our finding that GALT lymphocytes, and in particular intra-epithelial lymphocytes, exert natural and sIgA-dependent activity against an enteric pathogen further supports the hypothesis that cell-mediated responses of the type discussed above might be relevant as a first line of defence against infectious diseases. Apart from the obvious theoretical implications, these results could stimulate new approaches to obtain more rational and effective vaccines for gastrointestinal infections.

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1. Strober, W., Hague, N. E., Lum, L. G. & Henkart, P. A. *J. Immun.* **121**, 2440–2445 (1978).
2. Lum, L. G. *et al. J. Immun.* **122**, 65–69 (1979).
3. Lum, L. G., Muchmore, A. V., O'Connor, N., Strober, W. & Blaese, R. M. *J. Immun.* **123**, 714–719 (1979).
4. Fanger, M. W., Shen, L., Pugh, J. & Brenner, G. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3640–3644 (1980).
5. Stafford, H. A. & Fanger, M. W. *J. Immun.* **125**, 2461–2466 (1980).

6. Arnaud-Battandier, F., Hague, N. E., Lum, L. G., Elson, C. O. & Strober, W. *Cell. Immun.* **55,** 106–113 (1980).
7. Lowell, G. H., Smith, L. F., Artenstein, M. S., Nash, G. S. & MacDermott, R. P. *J. exp. Med.* **150,** 127–137 (1979).
8. Lowell, G. H. *et al. J. Immun.* **125,** 2778–2784 (1980).
9. Lowell, G. H., Smith, L. F., Griffis, J. M. & Brandt, B. L. *J. exp. Med.* **152,** 452–457 (1980).
10. Formal, S. B., La Brec, E. H., Kent, T. H. & Falkow, S. *J. Bact.* **89,** 1374–1382 (1965).
11. Nencioni, L., Villa, L., Boraschi, D., Berti, B. & Tagliabue, A. *J. Immun.* **130,** 903–907 (1983).
12. Keren, D. F. *Infect. Immunity* **24,** 441–448 (1979).
13. Arnaud-Battandier, F., Bundy, B. M., O'Neill, M., Bienenstock, J. & Nelson, D. L. *J. Immun.* **121,** 1059–1065 (1978).
14. Herberman, R. B. (ed.) *Natural Cell-mediated Immunity Against Tumours* 1321 pp. (Academic, New York, 1980).
15. Kreger, B. E., Craven, D. E., Carling, P. C. & McCabe, W. R. *Am. J. Med.* **68,** 332–343 (1980).
16. Tagliabue, A., Luini, W., Soldateschi, D. & Boraschi, D. *Eur. J. Immun.* **11,** 919–922 (1981).
17. Tagliabue, A., Befus, A. D., Clark, D. A. & Bienenstock, J. *J. exp. Med.* **155,** 1785–1796 (1982).
18. Herberman, R. B. *Clin. Immun. Rev.* **1,** 1–65 (1981).
19. Timonen, T., Ortaldo, J. R. & Herberman, R. B. *J. exp. Med.* **153,** 569–582 (1981).
20. Luini, W., Boraschi, D., Alberti, S., Aleotti, A. & Tagliabue, A. *Immunology* **43,** 663–668 (1981).
21. Finney, D. J. *Statistical Methods in Biological Assay* 2nd edn, 99–139 (Griffin, London, 1971).
22. Potter, M. *Physiol. Rev.* **52,** 631–719 (1972).

Role for mouse macrophage IgG Fc receptor as ligand-dependent ion channel

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The interaction of ligands with the mouse macrophage Fc receptor which binds IgG2b and IgG1 immune complexes ($FcR_{\gamma 2b/\gamma 1}$) triggers phagocytosis and secretion of various mediators of inflammation[1–4]. $FcR_{\gamma 2b/\gamma 1}$ has been purified using a monoclonal anti-FcR antibody, 2.4G2 (refs 5, 6), and seems to be an integral membrane glycoprotein of molecular weight (M_r) 47,000–60,000 (ref. 6). Monoclonal antibody 2.4G2 is suitable as a tool for functional studies of FcR because it binds to a functional site of the receptor and induces cellular responses that are normally associated with the occupied receptor[5,7,8]. We reported previously that binding of ligands to the macrophage FcR resulted in Na^+/K^+ ion fluxes through the plasma membrane, and that similar ion fluxes were observed in proteoliposomes containing reconstituted FcR[9,10]. We have now incorporated FcR into planar lipid bilayers and report here that $FcR_{\gamma 2b/\gamma 1}$ forms ligand-dependent cation-selective ion channels, with a conductance of 60 pS in 1 M KCl and an average open channel lifetime of 250 ms. The conductance decays to baseline levels within a few minutes. These results suggest a receptor–ionophore model for the signalling of phagocytosis and inflammatory responses.

$FcR_{\gamma 2b/\gamma 1}$ purified as described previously[5,6], was reconstituted into lipid vesicles by detergent dialysis or detergent dilution[11]. The integration of $FcR_{\gamma 2b/\gamma 1}$ into liposomes was confirmed by isopycnic centrifugation (Fig. 1). To assay directly for $FcR_{\gamma 2b/\gamma 1}$ in gradient fractions, a monoclonal sandwich radioimmunoassay with ^{125}I-2.4G2 Fab as labelled probe was used[12].

FcR–proteoliposomes were incorporated into planar bilayers by transforming lipid monolayers containing FcR into stable bilayers[13]. Electrical measurements were made as elsewhere[14]. Monolayers were prepared by a detergent-dilution protocol[15–17] (Fig. 2). The larger size of the liposomes (>5,000 Å) formed by detergent dilution comparison with those formed by detergent dialysis[11,18] may facilitate the spontaneous formation of the lipid monolayer[16].

Bilayers formed from monolayers of phosphatidylcholine–cholesterol (either protein-free or containing FcR up to 5 μg per membrane) by the detergent-dilution method showed baseline conductance levels of <10 pS and a capacitance of 0.72 ± 0.06 μF cm^{-2}. These values were not changed by addition of ligands to protein-free bilayers, demonstrating that the ligands *per se* have no effect on membrane conductance. Addition of ligands to the *trans*, or FcR-free, side of the membrane resulted in no or a small increase in membrane conductance (Fig. 2). By contrast, when ligands were added to the *cis* side, a dramatic increase in membrane conductance was observed, the magnitude of which depended on the concentrations of both the receptor and the ligand used (Fig. 2). This sidedness in response suggested that FcR oriented in a vectorial fashion during monolayer→bilayer assembly or that denaturation of the receptor with the 'wrong' orientation occurred at the air–water interphase. An increase in conductance was observed with voltages of both polarities.

The conductance increase could be activated by a variety of ligands specific for FcR (Fig. 2). Insoluble immune complexes gave a larger response than soluble complexes or 2.4G2 IgG, which was more effective than 2.4G2 Fab. Controls in which monomeric anti-dinitrophenyl DNP IgG was added to the *cis* side showed no conductance changes. These results indicate that the state of aggregation of FcR by ligands bears directly on the conductance changes observed. These observations mirror the response of intact macrophages to immune complexes in the triggering of release of mediators of inflammation[19].

The ligand-dependent increase in conductance always declined to baseline levels after a few minutes and was not reversed by subsequent addition of excess ligand (Fig. 2*a*). This inactivation or desensitization suggests that the perturbation of the receptor by ligand results in transient conformational change of the receptor followed quickly by the relaxation of the receptor to a new unresponsive state that will not allow ions to pass through the membrane.

Single FcR-channel fluctuations were obtained by reducing the amount of receptor in the bilayer. Figure 3 shows two such experiments, in which we used 2.4G2 IgG as ligand. The conductance changes always occurred in discrete fluctuations, which were indicative of opening and closing of single or groups of single channels. The responses observed in KCl and NaCl were similar. With a time resolution of 1 ms, we could not resolve the risetime of individual steps. This represents a flux over 10^6 ions per s per molecule, which would be much too fast for any type of transport mechanism other than an ion-channel mechanism[20,21].

To resolve single-channel fluctuations, the ionic strength of the bathing buffer was increased (Fig. 4). The conductance per channel was 60 ± 5 pS (1 M KCl) and 54 ± 6 pS (1 M NaCl) and the average lifetime of the open channel (within the first 5 min of addition of ligands) was 250 ms. No intermediate states were observed. Single-channel fluctuations were difficult to maintain because of the spontaneous inactivation of the conductance over time, the frequency of the open state decaying in an exponential fashion after the first few minutes of contact with ligand. In 1 M KCl, the current–voltage (I–V) plot for single channels was linear, indicating ohmic behaviour (Fig. 5). Thus, the inactivation or desensitization of the $FcR_{\gamma 2b/\gamma 1}$ is probably not due to a variable conductance of the channel itself but rather to a decreased frequency of opening of the bound receptor–channel. The kinetics of the channel depended on the lipid composition (and thus fluidity) of the bilayer, the open lifetime being shorter when lipids extracted from macrophages were used to form the bilayer.

The ion selectivity of the FcR–channel triggerred by 2.4G2 IgG was determined from the reversal potential necessary to null current flow from a 10-fold higher KCl or NaCl concentration in the *cis* compartment and was calculated using the Nernst–Planck relationship[17]. Because of the rapid spontaneous inactivation of conductance increase following addition of ligands, such experiments could not be performed on a

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