Arachidonic acid activatable electrogenic H^+ transport in the absence of cytochrome b_{558} in human T lymphocytes

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Abstract To test the suggested structural relationship between the electrogenic H^+ transporting system and the NADPH oxidase of phagocytes, the existence of the enzyme and the transport process was investigated in human tonsillar T lymphocytes. It is shown that tonsillar T cells possess an arachidonic acid activatable, Cd^{2+}- and Zn^{2+}-sensitive electrogenic H^+ efflux pathway with similar properties as reported earlier in various phagocytic cells. The presence of cytochrome b_{558}, the membrane component of the oxidase, could not be detected in tonsillar T lymphocytes either by immunoblot or by flow cytometric analysis. It is suggested that the electrogenic H^+ transporting pathway is structurally independent of the NADPH oxidase complex.

Key words: Electrogenic H^+-transporter; NADPH oxidase; Arachidonic acid; Phospholipase A_2; Neutrophil granulocyte; T lymphocyte

1. Introduction

A Cd^{2+} and Zn^{2+} inhibitable electrogenic H^+ conducting pathway has been described in the plasma membrane of different leukocytes, namely in human and pig neutrophil granulocytes, peripheral lymphocytes, macrophages and HL60 cells [1–11]. In electrophysiological studies a voltage-dependent, outwardly rectifying and absolutely H^+-selective current was characterized in various phagocytic cells [6–8,11]. Exogenous arachidonic acid (AA) and lowering of the intracellular pH were reported as the most common stimuli of the transport [4–11]. Recently also receptor-mediated activation of the H^+-efflux has been described in neutrophils [12,13].

An important physiological role for this H^+-conductance has been suggested in $O_2^{\bullet-}$-producing cells [1,2]. The $O_2^{\bullet-}$-generating NADPH oxidase consists of a membrane component (cytochrome b_{558}) and at least three cytosolic proteins ($p47_{phox}$, $p67_{phox}$ and rac) which are translocated to the membrane upon stimulation [14]. The enzyme transfers one electron from intracellular NADPH to extracellular (or intraphagosomal) O_2, thereby producing $O_2^{\bullet-}$ and H^+ in the external (or phagosomal) and in the cytoplasmic space, respectively. H^+-efflux through a channel could provide both charge compensation for the electron transport and prevent significant intracellular acidification during the respiratory burst.

Although H^+-transport through this pathway has been extensively characterized, the molecular basis of the proposed channel activity could not be identified, yet. The molecular and functional relationship between the NADPH oxidase and the putative H^+-channel was investigated in several studies. First, alteration of the electrogenic H^+-transport was observed in neutrophils of CGD patients lacking different components of the NADPH oxidase [15,16]. In a previous study we showed that in peripheral pig lymphocytes, which possess an electrogenic H^+-transporter in many aspects similar to that of granulocytes and macrophages, the two specific cytosolic components of the oxidase are not detectable. This finding indicates that the operation of the H^+-channel does not require the assembly of a functional NADPH oxidase [11]. On the basis of measurements carried out on CHO and undifferentiated HL60 cells transfected with the cDNA of $gp91_{phox}$, the large subunit of cytochrome b_{558}, Henderson et al. [17] suggested that the $gp91_{phox}$ protein could function as the AA activatable H^+-channel. The functional characterization of the expressed transport route remained, however, insufficient. The H^+-fluxes have not been investigated in the physiological outward direction which is the only possible direction of the transport according to several studies [6–8,10], neither was the sensitivity of the detected H^+-movements to heavy metals demonstrated.

In order to further investigate the possible involvement of the cytochrome b_{558} oxidase component in the H^+-channel activity, we examined the existence of an electrogenic H^+-transporting pathway in tonsillar T cells. This lymphocyte population is unable to produce $O_2^{\bullet-}$ and we presumed that they do not express cytochrome b_{558}. Our results suggest that the AA activatable and Cd^{2+},Zn^{2+}-sensitive electrogenic H^+-efflux in T cells is not coupled to the molecular presence of the cytochrome b_{558} protein.

2. Materials and methods

2.1. Chemicals and solutions

BCECF/AM and nigericin were obtained from Calbiochem; NDGA, AA, RPMI 1640 and AET from Sigma; valinomycin from Serva; Percoll from Pharmacia; FCS from Sebac, Lymphoprep from Nycomed. The FITC-labelled anti CD3 antibody was a kind gift from Dr. Katalin Pálóczi. The antibodies reacting with the small and large subunits of cytochrome b_{558} (anti-$p22_{phox}$ and $gp91_{phox}$) were kind gifts from Dr. Arthur J. Verhoeven and Prof. Dirk Roos [18].

Composition of the media used: *Phosphate-buffered saline (PBS) medium* = 137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4 and 5 mM glucose, pH 7.3; *KCl medium* = 140 mM KCl, 5 mM Tris-HCl and 5 mM glucose, pH 7.4; *RPMI medium* = RPMI 1640, 2 mM glutamine, 1 mM sodium pyruvate, 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% FCS; *FACS medium* = 132 mM

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Abbreviations: AA, arachidonic acid; BCECF/AM, 2′,7′-bis(carboxyethyl)-5(6)-carboxyflourescein acetoxymethyl ester; CGD, chronic granulomatous disease; CHO, Chinese hamster ovary; DCCD, *N,N′*-dicyclohexylcarbodiimide; NDGA, nordihydro-guaiaretic acid; PBS, phosphate-buffered saline; PLA_2, phospholipase A_2; pH_i intracellular pH; pH_o, extracellular pH; PE, *R*-Phycoerythrin; SDS-PAGE, SDS-polyacrilamide gel electrophoresis; AET, 2-aminoethyl-isothiouronium.

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NaCl, 6 mM KCl, 1.2 mM K_2HPO_4, 1 mM $MgSO_4$, 20 mM HEPES, 1 mM EGTA and 0.5% BSA.

2.2. Cell preparation

Lymphocytes were collected from human tonsils by standard techniques [19]. T cells were separated by rosetting with AET-bromide-treated sheep red blood cells [20]. Resting B cells were prepared according to [20].

Separated T cells (in rosettes) were suspended in RPMI medium and centrifuged through 65% Percoll in order to remove B cell contamination. T cells were collected from the bottom of the tube. To 2.5 ml of sample of this preparation 5 ml of 150 mM NaCl, 50 μl of 0.5 M Tris-EGTA and 15 ml of distilled water were added for lysis of erythrocytes. After 40 s of careful mixing, iso-osmotic conditions were restored with NaCl. Cells were pelleted (840×*g*, 10 min), washed and resuspended in PBS. Purity of the T cells and contamination of the B cell preparation with T cells was controlled by flow cytometric analysis using anti-CD3 antibody [21]. Purity of the preparations was 95–99%.

Neutrophil granulocytes were isolated from citrated human blood as described in [22].

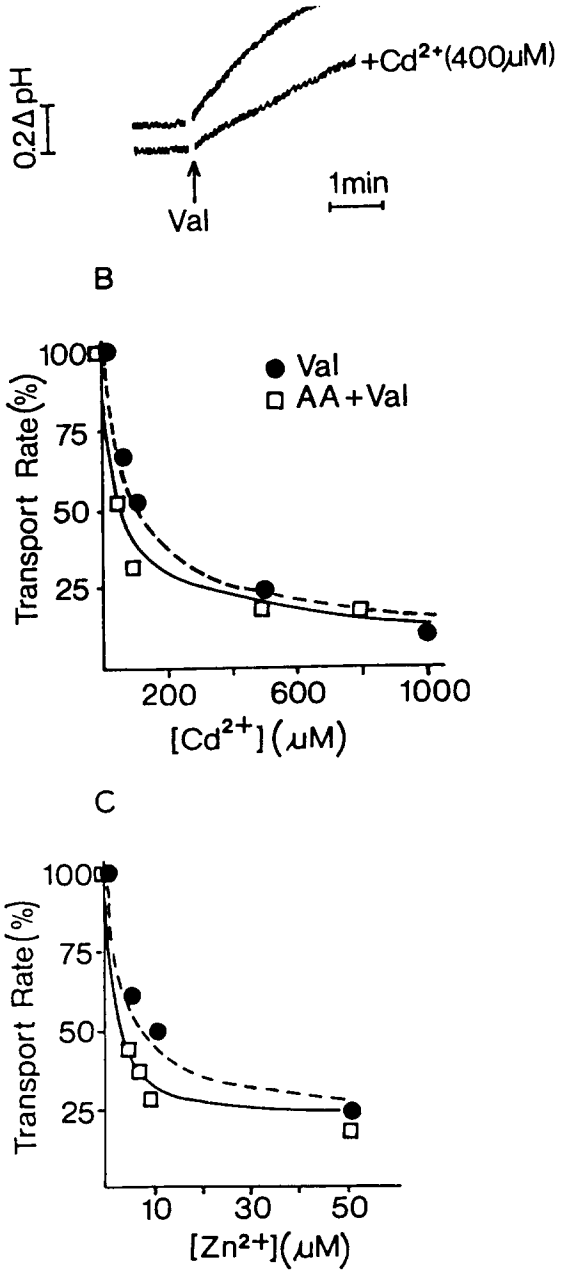

Fig. 1. Inhibition of the electrogenic H^+-transport by Cd^{2+} and Zn^{2+}. (A) Effect of Cd^{2+} on the valinomycin-induced intracellular alkalinization (one experiment, representative of five similar ones). (B and C) Inhibitory effect of different concentrations of Cd^{2+} (B) or Zn^{2+} (C) on the rate of H^+-extrusion from acidified tonsillar T cells under basal conditions (i.e. presence of valinomycin only) (●) or stimulated by 3 μM AA (□). The graphs show one experiment representative of three similar ones. The value 100% corresponds to 0.177 ΔpH/min or to 0.531 ΔpH/min in the case of basal or AA-stimulated H^+ efflux, respectively.

2.3. Measurement of H^+-movements through the plasma membrane of T and B cells

H^+-movements through the plasma membrane of T and B lymphocytes prepared from human tonsils were measured by monitoring intracellular pH changes with the same technique as used for the characterization of the electrogenic H^+-transporting pathway of neutrophil granulocytes, peripheral lymphocytes, macrophages and enterocytes [4,7,11,16,23]. Briefly, intracellular pH was measured on the basis of the fluorescence of the pH indicator dye BCECF. T or B cells (5×10^6–10^7) suspended in 1 ml of PBS were incubated with 4 μM BCECF/AM at 37°C for 20 min. Cells were then diluted, washed and resuspended at $5–7\times10^8$/ml in PBS medium. In order to obtain a transmembrane pH gradient of about 0.7 unit, the intracellular compartment of BCECF-loaded cells was acidified to pH_i 6.70 ± 0.14 ($n=4$) by using the ammonium pre-pulse technique [24] as detailed in [4]. Cells were finally resuspended in 150 mM choline chloride, 5 mM Tris-HCl and 5 mM glucose, pH 7.4. Acidified cells were kept at 4°C. Fluorescence of BCECF loaded cells was monitored by a Perkin-Elmer 3000 spectrofluorimeter with wavelengths of 495 and 530 nm for excitation and emission, respectively. No significant change in the baseline fluorescence was observed up to 3 h from the time of loading the cells with the indicator. Typically, 2.5×10^5 BCECF loaded cells were suspended in 2.5 ml of a KCl-based, Na^+-free medium (KCl medium). The high external K^+ concentration largely dissipated the internal negative membrane potential that could hinder the movement of protons. The lack of external Na^+ excluded the participation of the Na^+/H^+ exchanger in the pH_i changes. The possibility of the involvement of a K^+/H^+ exchanger in the detected H^+-transport was excluded as detailed previously [11]. The electrogenic H^+-efflux was initiated by the addition of 0.8 μM valinomycin. K^+ influx via this ionophore could serve as charge compensation for the proton extrusion through the channel. All further additions are detailed in the legend to the respective figure. Calibration of the signal in terms of pH_i was carried out as described in [4].

Experimental data are presented as mean ± S.E.M. of the number of experiments (n).

2.4. Immunodetection of the cytochrom b_{558} component of the NADPH oxidase

Lymphocytes and granulocytes (5×10^5 cells) were lysed in hot sample buffer containing 2% SDS and subjected to SDS-PAGE. The separation gel contained 10% acrylamide. Electrophoretic separation, transfer to nitrocellulose sheets and reaction with the antibodies were carried out as detailed in [25]. Alkaline phosphatase-labelled anti-mouse IgG was used as the second antibody and the colour reaction was developed with 5-bromo-4-chloro-3-indolyl phosphate plus nitro-bluetetrazolium.

2.5. Flow cytometry

Binding of the antibodies to permeabilized cells was carried out as described in [18]. Briefly, 2×10^6 neutrophils or tonsillar T cells were permeabilized for 2 min in FACS medium supplemented with 15 μM digitonin, then diluted 5-fold with cold FACS medium and centrifuged. The cells were washed and fixed for 5 min by adding an equal volume of 1% paraformaldehyde. After washing in PBS, the cells were incubated with the monoclonal anti-$gp91_{phox}$ antibody in FACS medium at 37°C for 25 min. Subsequently the cells were washed, resuspended in FACS medium containing 2% BSA and PE-labelled goat anti-mouse Ig and incubated for another 20 min at room temperature. Cells were washed twice, then incubated for additional 20 min with FITC-labelled anti-CD3 antibody followed by a last washing. Fluorescence intensities were measured with a Facstar fluorocytometer (Becton Dickinson).

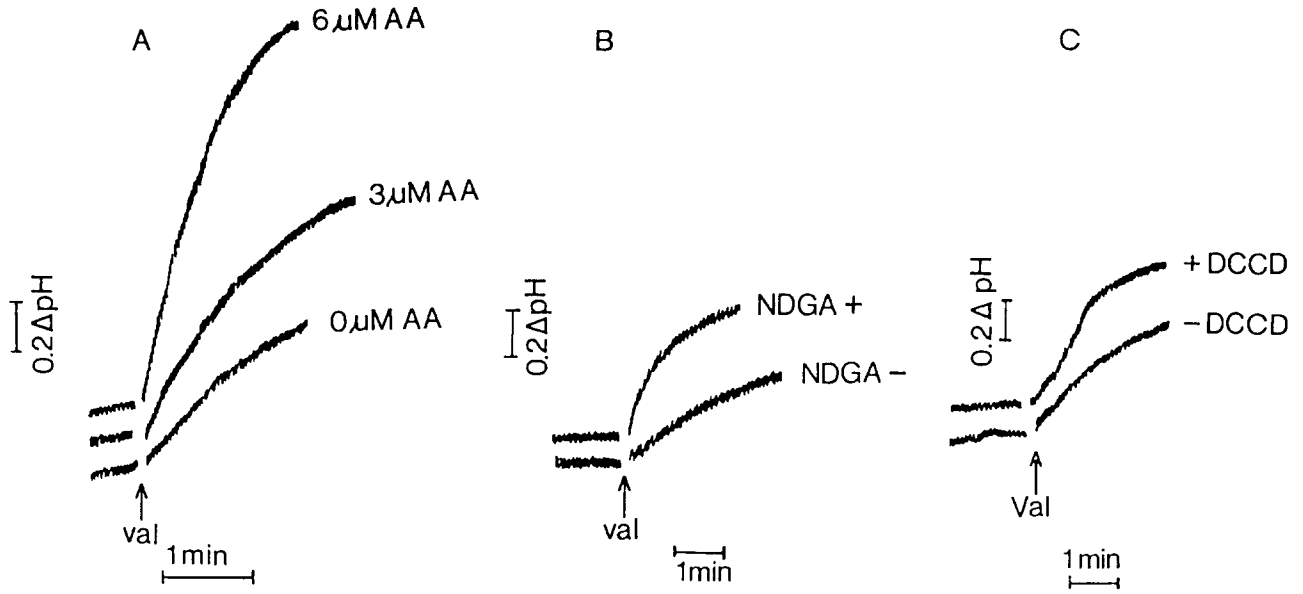

Fig. 2. Effect of different pharmacological agents on the electrogenic H^+-efflux from acidified tonsillar T cells. Effect of different concentrations of AA (A), or 25 μM NDGA (B) or 50 μM DCCD (C) on the valinomycin-induced H^+-transport. The respective agent was added before valinomycin. One experiment representative of four (A) or two (B) or three (C) similar ones is shown. For the clarity of presentation, the original traces were shifted vertically although the initial pH_i was similar.

3. Results

3.1. Characterization of an electrogenic H^+ transporting pathway in human tonsillar T lymphocytes

H^+-Movements through the plasma membrane of T lymphocytes prepared from human tonsils were measured as described in section 2. Fig. 1A shows our typical basic observation. After suspending the tonsillar T cells in the KCl based medium essentially no pH increase could be measured, whereas the addition of valinomycin induced a significant increase in the intracellular pH. The avarage rate of the initial pH_i-change was 0.177 ± 0.013 ΔpH/min ($n = 4$). The fact that the pH_i-change is promoted by the ionophore, i.e. by allowing charge compensating K^+ movement, suggests that the pH_i increase is the consequence of an electrogenic H^+-egress. Under comparable conditions, the initial rate of the electrogenic H^+-efflux from the T lymphocytes falls in the same range as reported earlier for neutrophil granulocytes, macrophages and peripheral lymphocytes [4,9,11].

Cd^{2+} and Zn^{2+} were described as common inhibitors of the electrogenic H^+-transporter of neutrophil granulocytes, macrophages and peripheral lymphocytes [1,2,4,6–11]. Fig. 1A shows that 400 μM Cd^{2+} causes a significant decrease in the initial rate of the valinomycin induced H^+-transport of tonsillar T cells. The curves with dotted lines in Fig. 1B and C represent the dependence of the initial rate of the valinomycin-induced (basal) transport on the concentrations of Cd^{2+} and Zn^{2+}. Half-maximal inhibition was attained at 125 μM Cd^{2+} and 9 μM Zn^{2+}, respectively. These values are close to those reported previously for neutrophil granulocytes [4] and peripheral lymphocytes [11] suggesting that similar transporters may function in these different cell types.

The available information about the pharmacological properties of the H^+ pathway is limited. However, in addition to the inhibitory effect of heavy metals, there are some common features characteristic for this route in granulocytes, macrophages and peripheral lymphocytes. These include the sensitivity to AA and various drugs influencing its metabolism as well as the activating effect of the carboxylating agent DCCD. These properties were tested in tonsillar T lymphocytes.

Fig. 2A shows the activation by exogenously added AA of the valinomycin induced H^+-efflux. Addition of AA at a concentration of 3 and 6 μM induced 2.91 ± 1.05- or 6.53 ± 2.1-fold ($n = 4$) increase in the transport rates, respectively. This effect of AA was strongly dependent on the presence of valinomycin indicating that AA does not aspecifically increase the permeability of the cells but probably stimulates the transport of protons through the electrogenic pathway. The H^+-movement activated by AA also proved to be Cd^{2+}- and Zn^{2+}-sensitive (Fig. 1B,C). The similar dependence of the rate of the basal (only valinomycin-induced) and the AA-activated transport on the concentrations of Cd^{2+} and Zn^{2+} suggests that the H^+-efflux occurs in both cases through the same transport molecule.

NDGA, an inhibitor of the AA metabolism through the lipoxygenase pathway has been described to activate the electrogenic H efflux from neutrophils [12]. As shown in Fig. 2B, a similar effect was observed in T lymphocytes: 25 μM NDGA caused a 5-fold elevation in the transport rate. On the other hand, inhibition of PLA_2 decreased the rate of H^+-efflux from phagocytes [9,12]. In T cells, 2 μM quinacrin, a potent inhibitor of the PLA_2 enzyme decreased the rate of the valinomycin-induced H^+-efflux by 40% and the NDGA-induced stimulation was also inhibited (data not shown). A plausible interpretation of these findings could be that NDGA activates and quinacrin inhibits the H^+-transport through influencing the level of the endogenously generated AA [26,27].

Finally, we tested the effect of DCCD on the T cell preparation. DCCD stimulated the Cd^{2+}-sensitive and valinomycin-induced electrogenic H^+-transport in a concentration dependent manner also in these cells. At a concentration of 50 μM, this compound increased the transport rate to $277 \pm 25\%$ of the basal activity ($n = 3$) (Fig. 2C). This value is near to that

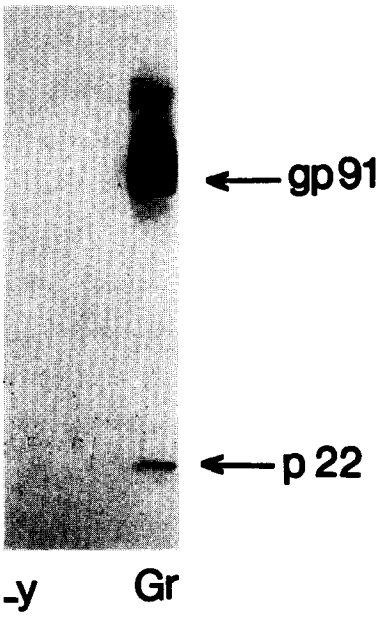

Fig. 3. Detection of cytochrome b_{558} by immunoblot. Same amounts (5×10^5) of tonsillar T cells (Ly) or peripheral granulocytes (Gr) were subjected to Western blot analysis as described in section 2. The large and small subunits of cytochrome b_{558} were detected by monoclonal antibodies against $gp91_{phox}$ and $p22_{phox}$ proteins. One experiment representative of 4 similar ones.

found previously in peripheral lymphocytes [11] and neutrophils (Kapus, Suszták and Ligeti, unpublished observations).

3.2. *Investigation of the presence of cytochrome b_{558} in tonsillar T cells*

Henderson et al. [17] suggested, that the AA-stimulated electrogenic H^+-transport in neutrophil granulocytes is mediated by the $gp91_{phox}$ component of the NADPH oxidase. We raised the question, whether the electrogenic H^+-efflux measured in the human T cell preparation can be catalysed by this molecule, the expression of which has not yet been investigated in tonsillar T cells. We carried out both Western blot and flow cytometric analysis on the purified tonsillar T cell population and the results were compared to data obtained in parallel measurements on neutrophil granulocytes.

The immunoblot shown in Fig. 3 revealed that tonsillar T lymphocytes neither express the $gp91_{phox}$ nor the $p22_{phox}$ component of the cytochrome b_{558} in sufficient amount to be detectable by this technique.

Fig. 4 summarizes the data obtained by flow cytometry on tonsillar T lymphocytes (Fig. 4A) and neutrophil granulocytes (Fig. 4B). Both cell populations were double-stained with FITC-labelled antibodies against CD3 markers and anti-$gp91_{phox}$ antibodies followed by a PE-conjugated second antibody. As shown on the dot plots, 78% of the analysed granulocytes are localized in the upper left quadrant, indicating that the anti-$gp91_{phox}$ antibody is able to react with its antigen under the applied experimental conditions (Fig. 4B). This should be compared to the results obtained with T lymphocytes in which more than 80% of the investigated cells are found in the lower right quadrant of the plot (Fig. 4A). Thus, the CD3 positive T lymphocytes are not stained with the antibody against the $gp91_{phox}$ protein.

These results support our conclusion that the cytochrome b_{558} component of the NADPH oxidase is not expressed in human tonsillar T lymphocytes. Similar data were reported earlier for human peripheral T lymphocytes [28].

4. Discussion

We propose that human tonsillar T cells possess in their plasma membrane an electrogenic H^+-transporting pathway, the properties of which resemble those of phagocytic cells and peripheral lymphocytes. We detected: (1) similar transport rates under basal conditions; (2) comparable inhibition by Cd^{2+} and Zn^{2+} (similar K_i values); (3) similar activation by AA and DCCD. According to our immunoblot and flow cytometric experiments tonsillar T cells lack the cytochrome b_{558} protein, thus it cannot be the structural basis of the electrogenic H^+-extrusion in these cells.

Our T lymphocyte preparation contained as a contamination 1–5% B cells. Human peripheral B cells are known to

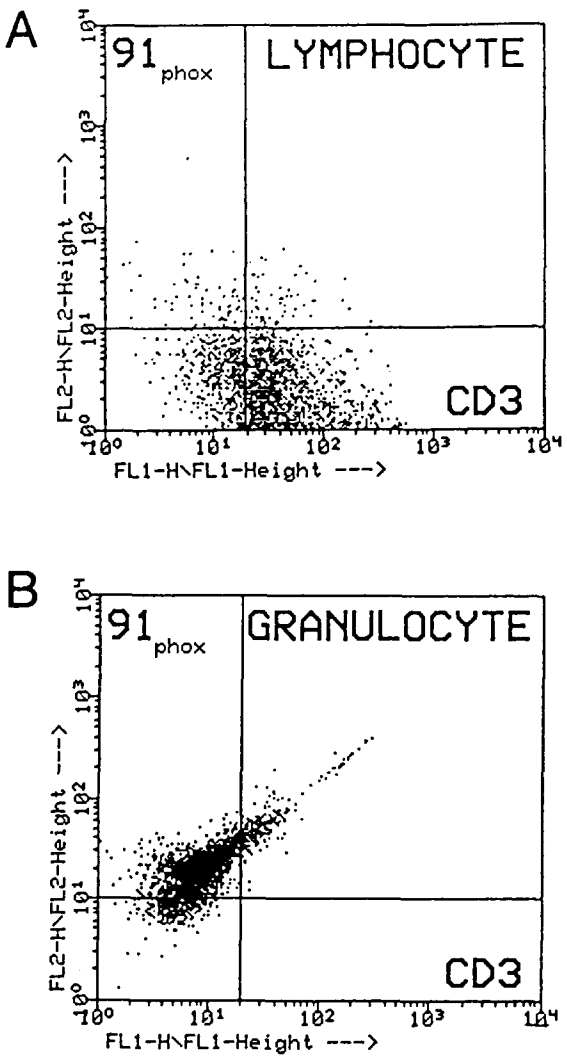

Fig. 4. Flow cytometric analysis of the expression of CD3 marker and $gp91_{phox}$ in tonsillar T cells (A) and peripheral granulocytes (B). Two-dimensional flow cytometric dot plots correlating the expression of CD3 (x-axis) and $gp91_{phox}$ (y-axis). The intensity of green fluorescence of FITS (x-axis) and of red fluorescence of PE (y-axis) are represented in arbitrary units, on a logarithmic scale. 96% of the control granulocytes and 89% of the lymphocytes fall in the left lower quadrant.

contain cytochrome b_{558} [28]. In order to exclude the possibility that the H^+-efflux measured on our preparation originates from B cells, we investigated H^+-movements also in purified B cell preparations. Under the same conditions, we detected a similar (electrogenic, Cd^{2+}- and Zn^{2+}-sensitive, AA- and DCCD-activatable) H^+ transporting system in tonsillar B cells as well (data not shown). However, the transport rates both under basal conditions and upon activation by AA are only 30–40% (e.g. basal activity = 0.0524 ± 0.0148 ΔpH/min; $n = 5$) of that calculated for T cells. In fact, any contamination with B cells would only decrease the rate of H^+ movement measured in the T lymphocyte preparation. These data clearly show that there is no correlation between the activity of the H^+ pathway and the presence of the cytochrome b_{558}.

H^+-Transporting systems similar to those described in leukocytes have been recently characterized in functionally different cell types, e.g. alveolar epithelial cells, oocytes, enterocytes and muscle cells [23,29]. Ability of these cells to produce superoxide has never been published, neither was the existence of $gp91_{phox}$ demonstrated. It is doubtful whether a protein highly specific for phagocytic cells could be responsible for the electrogenic H^+-movements of embryologically so distinct cell types. Finally, our data are in good agreement with recent findings on CGD patients' neutrophils, in which a H^+ conductance activated by depolarisation and internal acidification has been detected despite of absence of $gp91_{phox}$ [30].

Taken together, we suggest that the electrogenic H^+ transporting pathway is structurally independent of the NADPH oxidase complex. Nevertheless it can not be excluded that in phagocytic cells the oxidase may exert a direct or indirect regulatory effect on the activation of the putative H^+ channel as suggested by Nanda et al. [30].

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References

[1] Henderson, L.M., Chappell, J.B. and Jones, O.T.G. (1987) Biochem. J. 246, 325–329.
[2] Henderson, L.M., Chappell, J.B. and Jones, O.T.G. (1988) Biochem. J. 251, 563–567.
[3] Nanda, A. and Grinstein, S. (1991) Proc. Natl. Acad. Sci. USA 88, 10816–10820.
[4] Kapus, A., Szászi, K. and Ligeti, E. (1992) Biochem. J. 281, 697–701.
[5] Henderson, L.M. and Chappell, J.B. (1992) Biochem. J. 283, 171–175.
[6] DeCoursey, T.E. and Cherny, V.V. (1993) Biophys. J. 65, 1590–1598.
[7] Kapus, A., Romanek, R., QuYi, A., Rotstein, O.D. and Grinstein, S., (1993) J. Gen. Physiol. 102, 729–760.
[8] Demaurex, N., Grinstein, S., Jaconi, M., Schlegel, W., Lew, D.P. and Krause, K.H. (1993) J. Physiol. 466, 329–344.
[9] Kapus, A., Suszták, K. and Ligeti, E. (1993) Biochem. J. 292, 445–450.
[10] Kapus, A., Romanek, R. and Grinstein, S. (1994) J. Biol. Chem. 269, 4736–4745.
[11] Káldi, K., Szászi, K., Suszták, K., Kapus, A. and Ligeti, E. (1994) Biochem. J. 301, 329–33.
[12] Susztak, K., Káldi, K., Kapus, A. and Ligeti, E. (1995) FEBS Lett. 375, 79–82.
[13] Schumann, M.A., Leung, C.C. and Raffin, T.A. (1995) J. Biol. Chem. 270, 13124–13132.
[14] Morel, F., Doussiere, J. and Vignais, P.V. (1991) Eur. J. Biochem. 201, 523–546.
[15] Nanda, A., Grinstein, S. and Curnutte, J.T. (1993) Proc. Natl. Acad. Sci. USA 90, 760–764.
[16] Nanda, A., Curnutte, J.T. and Grinstein, S. (1994) J. Clin. Invest. 93, 1770–1775.
[17] Henderson, L.M., Banting, B. and Chappell, J.B. (1995) J. Biol. Chem. 270, 5909–5916.
[18] Verhoeven, A.J., Bolscher, B.G.J.M., Meerhof, L.J., Zwieten, R., Keijer, J., Weening, R.S. and Roos, D. (1989) Blood 73, 1686–1694.
[19] Boyum, A. (1968) Scand. J. Clin. Lab. Invest. 21 (Suppl.) 97, 51–76.
[20] Aman, P., Ehlin-Henrikssen, B. and Klein, G. (1984) J. Exp. Med. 159. 208–220.
[21] Sármay G., Rozsnyay, Z., Koncz, G., Danilkovich, A. and Gergely, J. (1995) Eur. J. Immunol. 25, 262–268.
[22] Geiszt, M., Káldi, K., Szeberényi, B.J. and Ligeti, E. (1995) Biochem. J. 305, 525–528.
[23] Peral, M.J. and Ilundiain, A.A. (1995) J. Physiol. (London) 484, 165–172.
[24] Swallow, C.J., Grinstein, S. and Rotstein, O.D. (1988) J. Biol. Chem. 263, 19558–19563.
[25] Ligeti, E., Pizon, V., Wittinghofer, A., Gierschik, P. and Jakobs, K.H. (1993) Eur. J. Biochem. 216, 813–820.
[26] Le-Gouvello, S., Colard, O, Theodorou, I., Bismuth, G., Tarantino, N. and Debre, P. (1990) J. Immunol. 144, 2359–2364.
[27] Cifone, M.G., Cironi, L., Santoni, A. and Testi, R. (1995) Eur. J. Immunol. 25, 1080–1086.
[28] Kobayashi, S., Imajoh-Ohmi, Sh., Kuribayashi, F., Nunoi, H., Nakamura, M. and Kanegasaki, S. (1995) J. Biochem. 117, 758–765.
[29] DeCoursey, T.E. and Cherny, V.V. (1994) J. Membrane Biol. 141, 203–223.
[30] Nanda, A., Romanek, R., Curnutte, J.T. and Grinstein, S. (1994) J. Biol. Chem. 269, 27280–27285.