The CD11c antigen couples concanavalin A binding to generation of superoxide anion in human phagocytes

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We have found that an anti-CD11c monoclonal antibody (MAb) inhibits the respiratory burst induced in phorbol 12-myristate 13-acetate (PMA)-differentiated U937 cells as well as in human peripheral blood monocytes and neutrophils upon cell stimulation with concanavalin A. The MAb had no effect, however, when the added stimulus was fMet-Leu-Phe or PMA. Flow cytometry analyses indicated that concanavalin A was able to interact with CD11c. The anti-CD11c MAb inhibited significantly concanavalin A binding to differentiated U937 cells, and concanavalin A blocked binding of anti-CD11c MAb to the cells. Binding of labelled concanavalin A to membrane proteins which were separated by PAGE and transferred to nitrocellulose paper indicated that proteins with apparent molecular masses similar to those of CD11c (150 kDa) and CD18 (95 kDa) molecules were the main concanavalin A-binding proteins in differentiated U937 cells as well as in mature neutrophils. Similar experiments carried out in the presence of the anti-CD11c MAb showed a specific and significant inhibition of concanavalin A binding to the CD11c molecule. These results indicate that concanavalin A binds to the CD11c molecule and this binding is responsible for the concanavalin A-induced respiratory burst in PMA-differentiated U937 cells as well as in human mature monocytes and neutrophils.

INTRODUCTION

The U937 cell line is a human monocyte/macrophage cell line derived from a histiocytic lymphoma which shares many characteristics of normal human peripheral blood monocytes [1]. U937 cells are susceptible to acquiring the phenotypic characteristics of mature monocytes and macrophages following exposure to several agents, including phorbol esters, γ -interferon and 1 α ,25-dihydroxyvitamin D₃ [2–5]. Phorbol esters are amongst the most effective differentiating agents and rapidly induce many morphological, biochemical and metabolic changes [4–7]. One of these phorbol-ester-induced changes is the acquisition of the capacity of differentiated U937 cells to generate superoxide anion [8,9]. Concanavalin A (Con A), a poor respiratory burst stimulus in mature neutrophils and monocytes, induces an elevated oxidative response in phorbol 12-myristate 13-acetate (PMA)-differentiated U937 cells [9]. Interestingly, the expression of the CD11b and CD11c antigens at the cell surface is induced in U937 cells after differentiation with PMA [5], concomitant with the acquisition of the capacity to generate superoxide anion upon addition of different stimuli [9] as well as with an increase in the Con A-binding capacity of the cells [10].

Monoclonal antibodies (MAbs) specific for the CD11c antigen were found to inhibit the production of superoxide anion in PMA-differentiated U937 cells stimulated with Con A [11]. This antigen corresponds to the α -subunit (150 kDa) of gp150,95, which belongs to the same family as the lymphocyte-function-associated antigen (LFA-1), and C3bi receptor (Mo1 or CR3) [12,13]. These antigens are composed of non-covalently linked α : β heterodimers which have distinct α -subunits and a common β -subunit of 95 kDa (CD18) [12,14–16]. The molecular masses of the distinct α -subunits are: 177 kDa (α -subunit of LFA-1 or CD11a), 165 kDa (α -subunit of Mo1; CD11b) and 150 kDa (α -subunit of gp150,95; CD11c) [12]. The anti-CD18 MAb has been reported to produce a slight inhibition of the Con A-stimulated

respiratory burst in PMA-differentiated U937 cells, whereas an anti-CD11b MAb was ineffective [11].

In the present report, we have found that the anti-CD11c MAb HC1/1 inhibits the Con A-stimulated oxidative response in PMA-differentiated U937 cells by inhibiting the binding of Con A to the CD11c antigen, indicating that Con A-stimulated superoxide anion generation is mainly due to the specific interaction of Con A with the CD11c antigen. Furthermore, we found similar results in human peripheral blood monocytes and neutrophils.

MATERIALS AND METHODS

Materials

RPMI 1640, fetal calf serum and L-glutamine were purchased from Flow Laboratories (Irvine, Scotland, U.K.). Antibiotics were from Llorente (Madrid, Spain). fMet-Leu-Phe, PMA and Con A were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). PMA was dissolved in dimethyl sulphoxide at a concentration of 2 mg/ml. This stock solution was stored in portions at –20 °C. Appropriate controls were performed to exclude an effect of dimethyl sulphoxide on cells. Con A was dissolved in distilled water at 2 mg/ml. Cytochalasin B, horse heart cytochrome c, superoxide dismutase and Hepes were purchased from Sigma. Protein A-Sepharose CL-4B was from Pharmacia (Uppsala, Sweden). Glucose oxidase and ionophore A23187 were purchased from Calbiochem (San Diego, CA, U.S.A.). Acrylamide, bisacrylamide, ammonium persulphate and NNN'-tetramethylethylenediamine (TEMED) were from Bio-Rad (Richmond, CA, U.S.A.). Na¹²⁵I was from Amersham International (Amersham, Bucks., U.K.). Lymphoprep was from Nyegaard and Co. A/S (Oslo, Norway) and protein molecular mass standards were from BRL (Gaithersburg, MD, U.S.A.).

Abbreviations used: Con A, concanavalin A; fMet-Leu-Phe, N-formyl-methionyl-leucyl-phenylalanine; MAb, monoclonal antibody; PMA, 4 β -phorbol 12-myristate 13-acetate.

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Cells

U937 cells were maintained in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum, 2 mM-glutamine, penicillin (100 units/ml) and gentamicin (24 µg/ml). Cells were incubated at 37 °C in a humidified atmosphere of CO₂/O₂ (1:19). Cell differentiation was induced by adding PMA to a final concentration of 35 ng/ml for 24 h [9].

Mature monocytes and neutrophils were isolated from fresh human peripheral blood obtained from adult donors by Lymphoprep centrifugation, after previous erythrocyte sedimentation with 1.3% dextran [17,18]. Remaining erythrocyte contamination of the neutrophil-enriched pellet was eliminated by hypo-osmotic lysis, resulting in a final cell population of more than 98% neutrophils, as assessed by Giemsa-Wright stain. The mononuclear cells were washed twice with phosphate-buffered saline (120 mM-NaCl and 2.7 mM-KCl in 10 mM-phosphate buffer, pH 7.6), and the cell suspension was added to Petri dishes to allow cells to adhere. After incubation at 37 °C for 2 h in a humidified atmosphere of CO₂/O₂ (1:19), the Petri dishes were washed six times with phosphate-buffered saline. The non-adherent cells were washed away and monocytes were collected with a rubber policeman.

Monoclonal antibodies

The MAb TS1/18, specific for the CD18 antigen [19], was kindly provided by Dr. T. A. Springer (Dana-Farber Cancer Institute, Boston, MA, U.S.A.). The anti-CD11c MAb was HC1/1 [20], which is highly specific for the CD11c antigen [20]. Hybridoma supernatants from each MAb were used in all the described experiments. P3X63 myeloma culture supernatant was used as negative control.

Superoxide anion generation

Release of superoxide anion was measured by the superoxide dismutase-inhibitable reduction of ferricytochrome *c* in a discontinuous assay as previously described [9]. PMA-differentiated U937 cells, monocytes and neutrophils were washed and resuspended in 1 ml of a glucose-containing buffer (10 mM-Hepes, 150 mM-NaCl, 1.2 mM-MgCl₂, 1.3 mM-CaCl₂ and 5.5 mM-glucose, pH 7.5). PMA-differentiated U937 cells, monocytes or neutrophils (5×10^5) were preincubated with 100 µl of the corresponding hybridoma supernatant for 15 min at 37 °C. Cells were washed with the glucose-containing buffer and then assayed for superoxide anion production in the presence of cytochalasin B by addition of the different stimuli (fMet-Leu-Phe, PMA, Con A) as previously described [9]. In the experiments described in Fig. 1, cells preincubated with the hybridoma supernatant were assayed for superoxide anion generation without washing out the excess MAb.

Unless otherwise indicated, the results given are the means ($\pm$ S.E.M.) for the numbers of experiments indicated.

¹²⁵I-labelling

Cell-surface labelling by lactoperoxidase/glucose oxidase-catalysed iodination with Na¹²⁵I was carried out as previously described [21].

Con A was ¹²⁵I-labelled by the chloramine-T method as previously described [22] by incubating 40 µg of Con A in the presence of 200 mM- α -methyl mannoside, 0.5 mCi of Na¹²⁵I and 10 µg of chloramine-T in 200 µl of phosphate-buffered saline.

Immunoprecipitation, PAGE and binding of ¹²⁵I-Con A to Transblot membrane proteins

Immunoprecipitation was performed as previously described [12,20]. Briefly, radiolabelled cells were lysed by incubation at

4 °C in a lysis buffer containing phosphate-buffered saline, pH 7.4, 1% Triton X-100, 1% haemoglobin and 2 mM-phenylmethanesulphonyl fluoride. After preclearing with 50 µl of 50% (v/v) Protein A-Sepharose in phosphate-buffered saline, labelled cell lysates were mixed at 4 °C with 100 µl of MAb hybridoma supernatant. After 2 h, 100 µl of 187.1 MAb hybridoma supernatant was added and the mixture was incubated overnight. To isolate immune complexes, 50 µl of 50% (v/v) Protein A-Sepharose in phosphate-buffered saline was added and incubation was continued for 2 h. Immunoprecipitates were washed twice with 0.1% Triton X-100 in phosphate-buffered saline, twice with phosphate-buffered saline and once with 10 mM-Tris/HCl, pH 6.8. Samples were subjected to SDS/PAGE on 7.5–15% (w/v) gradient gels or 10% gels [23] followed by autoradiography with enhancing screens [24].

Identification of the Con A-binding proteins was accomplished after blotting on to nitrocellulose paper (Millipore, 0.22 µm) of membrane proteins, previously separated by SDS/PAGE on 7.5–15% gradient gels or 10% gels, and incubation with ¹²⁵I-Con A (2×10^5 c.p.m./ml) for 3 h as previously described [25,26]. Controls run under the same conditions but in the presence of 40 mg of α -methyl mannoside/ml gave no bands in the corresponding autoradiograms. Densitometric analyses were performed in a Beckman DU-8D spectrophotometer.

Flow cytometry

Flow cytometry analysis was performed with an EPICS-C cytofluorimeter (Coulter Científica, Móstoles, Spain) as previously described [27]. Briefly, cells were incubated with the corresponding hybridoma culture supernatants (60 µl) for 30 min at 4 °C in a final volume of 200 µl of phosphate-buffered saline, washed twice with phosphate-buffered saline, and incubated for 30 min at 4 °C with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Kallestad Laboratories, Austin, TX, U.S.A.). Finally, cells were washed twice with phosphate-buffered saline and subjected to immunofluorescence flow cytometry analysis. In competition experiments cells were preincubated first with 5 µg of Con A/ml for 30 min at 4 °C, washed with phosphate-buffered saline and subjected to immunofluorescence analysis as above.

Flow cytometry analysis of Con A binding to the cells was performed by incubating 4×10^5 cells for 30 min at 4 °C with 2.75 µg of Con A, previously treated with fluorescein by the method described in [7]. After three washes with phosphate-buffered saline, the cells were analysed by flow cytometry. In competition experiments cells were preincubated first for 30 min at 4 °C with the corresponding hybridoma supernatants.

RESULTS

Effect of the anti-CD11c MAb on superoxide anion production in PMA-differentiated U937 cells, human monocytes and human neutrophils

Since it has been recently reported that the anti-CD11c MAb was able to inhibit the oxidative response in PMA-differentiated U937 cells [11], we examined whether this inhibitory effect was stimulus-dependent.

The effect of anti-CD11c MAb on the respiratory burst stimulated by different agents in PMA-differentiated U937 cells is shown in Table 1. Anti-CD11c MAb inhibited about 50% of the respiratory burst triggered by Con A, but no inhibitory effect was observed on the oxidative response stimulated by PMA or fMet-Leu-Phe. The inhibition by the anti-CD11c MAb was dose-dependent, as reported previously with dilutions of HC1/1 ascites fluid as well as with purified MAb [11]. Preincubation of PMA-differentiated U937 cells with the P3X63 myeloma super-

Table 1. Effect of the anti-CD11c MAb HC1/1 on the respiratory burst induced by different stimuli in PMA-differentiated U937 cells and in human neutrophils

PMA-differentiated U937 cells and human neutrophils were incubated with the negative control P3X63 (control) or with the anti-CD11c MAb HC1/1 for 15 min at 37 °C and assayed for $O_2^{\cdot -}$ production as described in the Materials and methods section. Values represent the means $\pm$ S.E.M. for three different determinations. In parentheses are the percentage activities referred to the amount of superoxide anion produced when cells were incubated with P3X63 (control; 100 %).

Stimulus	Superoxide anion production ^a (nmol of cyt. <i>c</i> reduced/10 min per 5×10^5 cells)			
	PMA-differentiated U937		Neutrophils	
	Control	HC1/1 (anti-CD11c)	Control	HC1/1 (anti-CD11c)
PMA (1 μ g/ml)	3.3 $\pm$ 0.3	3.6 $\pm$ 0.1 (109.1 %)	22.2 $\pm$ 2.3	21.2 $\pm$ 2.0 (95.5 %)
fMet-Leu-Phe (0.1 μ M)	2.2 $\pm$ 0.3	1.8 $\pm$ 0.3 (81.8 %)	11.2 $\pm$ 3.0	10.5 $\pm$ 2.8 (93.8 %)
Con A (50 μ g/ml)	15.4 $\pm$ 0.7	7.8 $\pm$ 0.6 (50.6 %)	1.2 $\pm$ 0.2	0.6 $\pm$ 0.1 (50 %)

Fig. 1. Effect of anti-CD11c MAb on Con A-induced superoxide anion generation in human peripheral blood neutrophils

Neutrophils (10^6) were incubated at 4 °C for 15 min with 100 μ l of the anti-CD11c MAb hybridoma supernatant. Then, cells were stimulated with the indicated Con A concentrations for 30 min at 37 °C and the superoxide anion production was monitored by the cytochrome *c* reduction assay as described in the Materials and methods section. Control cells in the absence of MAb were run in parallel. Data from control untreated cells ($\square$) and from anti-CD11c MAb-treated cells ($\blacksquare$) are shown as mean values $\pm$ S.E.M. of three independent experiments.

natant did not affect the respiratory burst induced by Con A or by the other activating agents in these cells (Table 1). Interestingly, the addition of anti-CD11c MAb also inhibited about 50 % the oxidative response elicited by Con A in human neutrophils, but had no effect on the neutrophil respiratory burst induced by PMA or fMet-Leu-Phe (Table 1). The inhibition by anti-CD11c of the respiratory burst induced by Con A in human neutrophils was almost complete when superoxide anion generation was assayed in cells pretreated for 15 min with 100 μ l of anti-CD11c MAb hybridoma supernatant and assayed as described in the Materials and methods section, but without washing the cells to eliminate the excess MAb (Fig. 1). Under these experimental conditions, anti-CD11c MAb was able to inhibit 80–90 % of the respiratory burst induced in human neutrophils by Con A in the range 1–100 μ g/ml (Fig. 1). Nevertheless, anti-CD11c MAb, assayed under the same experimental conditions, did not affect the neutrophil respiratory burst induced by 1000, 100, 10 or 1 ng of PMA/ml, or by 1 nM-, 10 nM-, 0.1 μ M- or 10 μ M-fMet-Leu-Phe. As previously

Fig. 2. Con A-binding proteins (a) and immunoprecipitation with anti-CD11c MAb (b) in PMA-differentiated U937 cells

(a) Membrane proteins (80 μ g) from PMA-differentiated U937 cells were separated by SDS/PAGE on 7.5–15 % acrylamide gradient gels and transferred to nitrocellulose paper. Then blots were incubated with 125 I-labelled Con A followed by autoradiography as described in the Materials and methods section. (b) PMA-differentiated U937 cells were surface-labelled with 125 I and immunoprecipitated with the anti-CD11c MAb HC1/1 as described in the Materials and methods section. Labelled antigen was analysed by SDS/PAGE on 7.7–15 % gradient gels. Bands with molecular masses of 150 kDa and 95 kDa, corresponding to the α - and β -subunits of the gp150,95 molecule, are indicated.

described [9], Con A-stimulated PMA-differentiated U937 cells produced much greater amounts of superoxide anion than Con A-stimulated neutrophils (Table 1). A similar inhibitory effect (50 % inhibition) by the anti-CD11c MAb on the respiratory burst induced by 50 μ g of Con A/ml in human peripheral blood monocytes was also observed (2.0 ± 0.4 versus 1.0 ± 0.3 nmol of cytochrome *c* reduced/10 min per 5×10^5 cells in untreated versus anti-CD11c-treated cells). However, anti-CD11c MAb did not inhibit the respiratory burst induced in peripheral blood monocytes by 1 μ g of PMA/ml or by 0.1 μ M-fMet-Leu-Phe (results not shown). These results indicate that the inhibitory action of the anti-CD11c MAb is not due to an effect on the general mechanism of superoxide anion generation, but to an effect on the specific activation of the respiratory burst by Con A.

Fig. 3. Con A-binding proteins (a) and immunoprecipitation with anti-CD11c MAb (b) in human peripheral blood neutrophils

(a) Membrane proteins (80 μ g) from human peripheral blood neutrophils were separated by SDS/PAGE on 10% acrylamide gels and then transferred to nitrocellulose paper. Blots were incubated with 125 I-labelled Con A followed by autoradiography as described in the Materials and methods section. (b) Human peripheral blood neutrophils were surface-labelled with 125 I and immunoprecipitated with the anti-CD11c MAb HC1/1 as described in the Materials and methods section. Labelled antigen was analysed by SDS/PAGE on 10% gels. The 150 kDa band, corresponding to the α -subunit of the gp150,95 molecule, is indicated.

Fig. 4. Effect of anti-CD11c and anti-CD18 MABs on Con A binding to PMA-differentiated U937 cells

U937 cells differentiated with PMA (35 ng/ml) for 24 h were preincubated for 30 min at 4°C in the presence of the negative control P3X63 (a), anti-CD11c MAB (b) or anti-CD18 MAB (c), before labelling with fluorescein isothiocyanate-conjugated Con A. Cells then were subjected to immunofluorescence flow cytometry as indicated in the Materials and methods section.

Binding of Con A to the CD11c antigen

A putative mechanism by which anti-CD11c MAB inhibits the Con A-stimulated oxidase activity could be the blockade of Con A-binding to a protein responsible for the functional cellular

Fig. 5. Effect of Con A on binding of anti-CD11c and anti-CD18 MABs to PMA-differentiated cells

U937 cells differentiated with PMA (35 ng/ml) for 24 h were preincubated in the absence (···) or in the presence (—) of 5 μ g of Con A/ml for 30 min at 4°C, before adding the corresponding MABs and labelling with fluorescein isothiocyanate-conjugated goat anti-mouse IgG. Then cells were subjected to immunofluorescence flow cytometry as described in the Materials and methods section. Profiles represent cells labelled with monoclonal antibodies specific for the following surface antigens: P3X63 negative control (a), CD11c (b) and CD18 (c).

response to Con A. To test this point, we examined the Con A binding to proteins separated by PAGE. As shown in Fig. 2, two main Con A-binding proteins in PMA-differentiated U937 cells possessed similar molecular masses to those of the α -subunit (CD11c) and the β -subunit (CD18) of the gp150,95 antigen [12]. CD11c and CD18 antigens were both immunoprecipitated with anti-CD11c HC1/1 MAB [20], as these antigens are associated to form the gp150,95 molecule [12]. In human neutrophils, the main Con A-binding protein had the same molecular mass as the CD11c antigen (Fig. 3).

Flow cytometry analyses indicated that anti-CD11c and anti-CD18 MABs decreased significantly the Con A binding to the PMA-differentiated U937 cells (Fig. 4). Conversely, Con A inhibited drastically the binding of anti-CD11c and anti-CD18 MABs to the cells as determined by fluorescence flow cytometry (Fig. 5). The percentage of PMA-differentiated U937 cells positive for CD11c ranged between 52 and 97% in the absence of Con A and between 13 and 35% in the presence of Con A.

To identify further the glycoproteins specifically affected by anti-CD11c MAB in relation to their capacity to bind Con A, we separated the membrane proteins from PMA-differentiated U937 cells by PAGE, blotted them on to nitrocellulose paper and incubated them with radioiodinated Con A in either the absence or the presence of anti-CD11c MAB (Fig. 6). It was observed that the Con A binding to a 150 kDa protein was drastically

Fig. 6. Effect of anti-CD11c MAb on Con A-binding proteins in PMA-differentiated U937 cells

Membrane proteins (80 µg) obtained from PMA-differentiated U937 cells were separated by SDS/PAGE on 10% gels, transferred to nitrocellulose paper and preincubated for 15 min with 100 µl of the negative control P3X63 (—) or 100 µl of anti-CD11c MAb (---), before incubation with 125 I-Con A as described in the Materials and methods section. The main bands affected by preincubation with anti-CD11c MAb are marked with numbers 1 and 2, corresponding to proteins of 150 kDa (CD11c) and 115 kDa. Scannings of the respective autoradiograms are shown. The molecular sizes were estimated by reference to the following protein markers: myosin (H-chain) (200 kDa), phosphorylase *b* (97.4 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), α -chymotrypsinogen (25.7 kDa), β -lactoglobulin (18.4 kDa) and lysozyme (14.2 kDa).

diminished when anti-CD11c MAb was present in the assay. This 150 kDa protein could be identified as the CD11c antigen, since anti-CD11c HC1/1 MAb reacted specifically with the 150 kDa CD11c molecule [20], as shown in Figs. 2 and 3. An additional band of about 115 kDa was also affected by anti-CD11c MAb with regard to its capacity to bind 125 I-Con A. This latter polypeptide could be a degradative product of the native CD11c antigen or a precursor molecule. These results clearly demonstrate that Con A is able to interact with the CD11c antigen.

DISCUSSION

The CD11c antigen has been implicated in adhesion-related processes such as migration, chemotaxis, phagocytosis, target-effector conjugate formation and C3bi binding [28–30]. Furthermore, the CD11c antigen has recently been shown to be involved in the production of superoxide anion by PMA-differentiated U937 cells stimulated by Con A [11]. Interestingly, we have previously found that U937 cell differentiation with PMA induced a high stimulation by Con A of the respiratory burst activity [9] as well as an increase in the Con A-binding capacity of the cells [10]. We also found evidence that only one or a small number of Con A-binding proteins are functionally involved in the Con A-induced respiratory burst in PMA-differentiated U937 cells [10]. In the present paper we have found that inhibition of the respiratory burst by the anti-CD11c MAb is dependent on the use of Con A as the stimulus and that specific Con A binding to the CD11c molecule was responsible for the Con A-triggered superoxide anion production in PMA-differentiated U937 cells. The specific inhibition by anti-CD11c MAb of the Con A-induced respiratory burst was also demonstrated in human peripheral blood monocytes and neutrophils. Nevertheless, anti-CD11c MAb did not affect the oxidative response elicited by either PMA or fMet-Leu-Phe in PMA-differentiated U937 cells, monocytes or neutrophils. Thus the superoxide anion

generation system itself is not affected by anti-CD11c MAb, as cells preincubated with anti-CD11c MAb are able to respond normally to stimuli other than Con A. We have found that the inhibitory effect of anti-CD11c MAb on the respiratory burst seems to be due to the prevention of Con A binding to the CD11c molecule. In support of this view is the finding that one of the major surface proteins that binds Con A in PMA-differentiated U937 cells is the gp150,95 antigen, in agreement with other reports in human neutrophils and alveolar macrophages [31–33].

Monoclonal antibodies against the β -subunit (CD18) of the gp150,95 antigen were found to affect Con A binding to the PMA-differentiated U937 cells in a similar way to the anti-CD11c MAb, as assessed by flow cytometry analysis. However, anti-CD18 MAb inhibited only 23% of the respiratory burst in these cells [11]. Thus the present results indicate that although Con A binds to many cell surface glycoproteins, the specific interaction of Con A with the CD11c molecule triggers an as yet unknown mechanism which directs superoxide anion generation. On these grounds, we propose a functional role for the CD11c molecule in the coupling of Con A binding with the triggering of the respiratory burst in human phagocytes. In this context, it is interesting to note that undifferentiated U937 cells lack CD11c antigen as well as lacking the capacity to generate superoxide anion, whereas PMA-differentiated U937 cells do contain CD11c and display a significant Con A-induced respiratory burst [5,9]. Furthermore, a correlation exists between the relative abundance of the CD11c molecule in distinct types of human phagocytes and their capacity to generate superoxide anion upon Con A stimulation. Thus values of the Con A-induced superoxide anion production by PMA-differentiated U937 cells, human monocytes and human neutrophils were 15.4, 2.0 and 1.2 nmol of cytochrome *c* reduced/10 min per 5×10^5 cells respectively, in agreement with previous estimates [9]. The relative abundance of CD11c antigen on the cell surface as determined by flow cytometry analysis in the distinct phagocyte populations was: PMA-differentiated U937 cells $\gg$ monocytes $\geq$ neutrophils [5].

The induction of the respiratory burst in human phagocytes by the specific interaction of Con A with CD11c is of major importance, since phagocytes may recognize bacteria by lectin-carbohydrate interactions in the absence of opsonins [34,35]. Furthermore, interactions between lectin-like proteins and carbohydrates seem to mediate physiologically significant adhesion of leucocytes with the vessel wall [36,37]. These interactions also seem to play a key role in cell recognition, controlling manifold normal and pathological processes in living organisms [38].

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