Lepr Rev (1993) **64,** 208–218

Modulation of peripheral blood derived monocytes/macrophages from leprosy patients using 'tuftsin' for production of reactive oxygen intermediates

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Accepted for publication 11 March 1993

Summary Phagocytic cells respond to a variety of membrane stimulants by producing reactive oxygen intermediates (ROI), i.e. O_2^-, H_2O_2 and OH· metabolites. Plasma membrane activation is associated with superoxide generating NADPH oxidase, thereby causing the production of these toxic species. Stimulation of phagocytic cells also results in activation of purine catabolism, which directs the metabolic flux through xanthine oxidase to produce the superoxide anion. We previously observed that BL/LL macrophages (Mϕ exhibited a premature inability to undergo tuftsin stimulated phagocytosis and microbicidal activity. The present study was undertaken to measure ROI levels in the absence and presence of 'tuftsin' pulsing as a function of *in vitro* culture age and also correlated these levels with adenosine deaminase (ADA) activity. The latter is known to be a contributor of O_2^- generation and is also involved in the maturation of the monocyte/macrophage system. The behaviour of normal and tuberculoid monocytes/macrophages were more or less the same, either in the presence or absence of tuftsin, i.e. they showed a progressive increase in ROI production until day 3, then tapered off in older cultures by day 7. In contrast, after day 1, the lepromatous macrophages were unable to undergo tuftsin mediated stimulation for the production of ROI and ADA activity. These findings indicate a defective Mϕ function in lepromatous patients towards tuftsin pulsing, thereby supporting our earlier observations. Thus BL/LL Mϕ behaved as if they were aged after 1 day of *invitro* culture, which may account for an inability to handle *Mycobacterium leprae* for efficient killing.

Introduction

Mononuclear phagocytes are indispensable to both natural and acquired host immunity. *M. leprae* is an obligate intracellular pathogen that is ingested by and proliferates within

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0305-7518/93/064208+11 $01.00

the cells of the monocytes/macrophages. The inability of macrophages in lepromatous leprosy (LL) patients to efficiently kill *M. leprae* is a predominant feature of this form of leprosy and consequently the macrophages in these patients are found to be loaded with *M. leprae*.

Phagocytic cells respond to a variety of membrane stimulants by the production and extracellular release of reactive oxygen intermediates (ROI), i.e. O_2^-, H_2O_2 and OH· metabolites. The production of these potentially active metabolites has been correlated with the intracellular killing of invading pathogens.[1,2] Recent studies have shown that intracellular pathogen may escape killing mechanisms either by inhibiting the production of ROI or by neutralizing these intermediates.[3] Phagocytosis or activation of plasma membrane enzymes by stimuli is initiated by the rapid consumption of molecular O_2 to form oxygen radicals by the NADPH oxidase system. Furthermore, the purine salvage pathway, xanthine oxidase, has also been implicated as an important contributor of O_2^- release by the phagocytic cells.[4,5] In this pathway adenosine deaminase (ADA) controls the amount of substrate (xanthine) available to xanthine oxidase, which is also required for normal immune function.[6]

In our previous study, the biphasic variation was observed during phagocytosis and the microbicidal response profile of monocytes/macrophages to 'tuftsin' stimulation against *Staphylococcus aureus*, *M. tuberculosis* and *M. leprae* as a function of culture age, corresponding to that seen with normal, BT/TT and BL/LL Mϕ led us to postulate aberrant maturation drives in BL/LL cultures (oxidative burst heterogeneity) of monocytes/macrophages.[7,8] This is further supported by low levels of endogenous serum tuftsin in lepromatous patients.[9]

In view of the aberrant phagocytic and microbicidal functions noticed in BL/LL culture, the present study was undertaken to measure the ROI production as a function of culture age after tuftsin pulsing and to correlate the results, with ADA activity, known to be involved in the maturation profile of monocytes/macrophages.

Materials and methods

PATIENTS

A total of 27 normal, 27 BT/TT and 22 BL/LL individuals, classified according to their clinical and histopathological findings, were assayed for ROI production and ADA activity. The patients were registered at the leprosy clinic, Department of Dermatovenereology, AIIMS, New Delhi. Patients received anti-Hansen's chemotherapy for less than 6 months in few cases, while most were fresh, untreated cases.

HUMAN MONONUCLEAR CELL VIABILITY AND PURITY

In brief, peripheral blood mononuclear cells (PBMC) were isolated by density gradient separation on Ficoll-paque. The interphase cells were collected, washed with cold Hank's balanced salt solution (HBSS) and suspended in RPMI-1640 (Gibco-Biocult) medium containing 10% human AB serum (heat-inactivated) containing the appropriate antibiotics, at a concentration of $1–2 \times 10^6$ cells/ml. This was then distributed equally into 24-well plates (Linbro, Flow Labs) and incubated for 3 h at 37°C in a moist atmosphere containing 5% CO_2. The non-adherent cells were removed by washing with pre-warmed

HBSS (37°C) and the monocyte enriched monolayers were then cultured in RPMI 1640-10% AB serum.[7] The adherent cells were removed at varying time intervals (1–7 days) by cold treatment and scraping with a rubber policeman. Before each assay, the adherent cells from the same individual were pooled, counted and tested for viability by tryphan blue exclusion. Routinely the yield of adherent cells was 10–15% of PBMC with a viability ranging from 95 to 97% and non-specific esterase positivity ranging from 90 to 95%. The number of adherent monocytes/macrophage were maintained at $0{\cdot}4 \times 10^5$ cells/100 μl/well in 96 well flat bottom plates for all subsequent experiments. All experiments were done twice and each sample was taken in duplicate wells.

SUPEROXIDE ANION ASSAY

Production of the superoxide anion was based on superoxide dismutase inhibitable reduction of ferric cytochrome C.[10] On the appropriate day of the assay, monocytes/macrophages were transferred to 96 well plates and were allowed to adhere for 2 h at 37°C in 5% CO_2 95% air. The media was removed and cells were washed with 0·1 ml of pre-warmed phenol red free HBSS to ensure uniform monocyte/macrophage monolayers on the bottom of the wells. The monolayers were covered with 80 μl of cytochrome C (4 mg cytochrome C/ml of phenol red free HBSS) and pulsed with tuftsin (0·22 μM and 0·88 μM). PMA (32 nM) was used as a positive stimulant. A replicate assay was performed in the presence of 20 μl of SOD (0·03 mg SOD/ml of HBSS) to verify the contribution of O_2^- to the reduction of cytochrome C. The reading was taken in an ELISA reader at 550 nm. Since the cells and cytochrome C solution in the plates were in no way disturbed by the measuring procedure, the plates were allowed to incubate for additional time intervals (30, 60 and 90 min) and reread. The amount of O_2^- produced was calculated from the extinction coefficient for the absorption of reduced cytochrome C, minus oxidized cytochrome C as read at 550 nm by the formula $21 \times 10^3\ M^{-1}\ cm^{-1}$. In the experimental conditions described above, the amounts of O_2^- produced per well, was expressed as follows:

$$\text{nanomoles } O_2^- \text{ per well} = \frac{\text{absorbance at 550 nm}}{6{\cdot}3} \times 100.$$

HYDROGEN PEROXIDE ASSAY

The assay of H_2O_2 production was based on HRPO-dependent oxidation of phenol red by H_2O_2 into a compound with increased absorbance at 600 nm.[10]

We added 100 μl/well of phenol red solution (PRS) to the cell monolayers. The PRS contained 140 mM NaCl, 10 mM potassium phosphate buffer, pH 7·0, 5·5 mM dextrose, 0·56 mM (0·2 g/L) phenol red and 19 U/ml of HRPO. Varying concentration of stimulant (tuftsin 0·22 μM and 0·88 μM) was added. PMA (32 nM) was used as positive control. Another set was kept without any stimulant. The blank was set up by putting an assay (without any cells) containing phenol red solution. The plates were incubated for the desired time period in humidified 5% CO_2–95% air chamber. Reaction was stopped with 10 μl of 1 N NaOH. The reading was taken in an ELISA reader at 600 nm. A standard curve for H_2O_2 was established for each plate using H_2O_2 ranging from 1 to 40 μM concentration with 100 μl of PRS and incubating for the desired time period at 37°C. The

amounts of H_2O_2 liberated by monocyte cultures was calculated through the standard curve.

ADENOSINE DEAMINASE ACTIVITY

Adenosine deaminase activity was measured essentially by the method of Tritsch *et al.*,[11] i.e. based on the rate of decrease in absorbance of adenosine at 265 nm.

The monocytes/macrophages monolayer was stimulated with tuftsin (0·22 μM and 0·88 μM). Another set of cells was treated with PMA (32 nM) as positive controls. One set of cells was kept without any stimulant. Cells were incubated for 1 hour. After the incubation, cells were lysed by adding 200 μl of chilled 0·5% triton X-100. The cells were scraped gently. The cell lysate was added to another tube containing 40 μl 1 mM adenosine, 60 μl tris buffer, 1·063 M, pH 7·3, and 100 μl of H_2O, and the decrease in absorbance at 265 nm was recorded. A change in 1·0 absorbance is considered to be equivalent to 0·13 μmol of adenosine deaminated.[12] Results are expressed as nmole ADA activity/hr/10^6 cells.

STATISTICAL ANALYSIS

All the experiments were done in duplicate and the results were expressed as the mean $\pm$ SD of the total number of patients in each group. Statistical analysis was done by Student's *t*-test.

Results

O_2^- PRODUCTION

The basal levels of O_2^- production on day 1 by monocytes of unstimulated cultures of normal and BL/LL subjects were within the same range (normal 0·57 $\pm$ 0·45 nmol/hr/10^6 cells, BL/LL 0·55 $\pm$ 0·41 nmol/hr/10^6 cells) while the basal level was higher in BT/TT Mϕ, i.e. 1·19 $\pm$ 0·35 nmol/hr/10^6 cells. When these cultures were stimulated with PMA (32 nM) and tuftsin (0·22 μM) they showed increased O_2^- production in normal and BT/TT Mϕ as compared to unstimulated monocytes ($p < 0·01$), whereas the BL/LL monocytes failed to show the response. However, PMA is able to stimulate O_2^- production in BL/LL monocytes (1·48 $\pm$ 0·25 nmol/hr/10^6 cells). At higher concentration of tuftsin (0·88 μM), normal and BT/TT Mϕ failed to show O_2^- release and the levels are the same as unstimulated cultures.

The 3-day-old cultures showed a progressive increase in O_2^- production in normal as well as in BT/TT macrophages upon stimulation, while BL/LL Mϕ almost showed the basal levels. In normal individuals, the 3-day-old Mϕ cultures showed a 3-fold increase in O_2^- production, by any of the stimulants (PMA 32 nM or tuftsin 0·22 μM) (Table 1); 3-day-old BT/TT Mϕ also showed approximately a 2-fold increase in O_2^- production by the same stimulants as compared to the unstimulated Mϕ of the same culture age. The basal level of O_2^- production was higher in BT/TT Mϕ as compared to normals (normal 0·49 $\pm$ 0·22 nmol/hr/10^6 cells, BT/TT 1·28 $\pm$ 0·45 nmol/hr/10^6 cells).

The 7-day-old Mϕ cultures of all groups under identical conditions behaved very

Table 1. Day and dose response profile for superoxide anion production by normal and leprosy monocytes/macrophages

Unstimulated	PMA (32 nM)	Tuftsin (0·22 μM)	Tuftsin (0·88 μM)
1-day-old culture			
N 0·57 ± 0·45	1·26 ± 0·72	0·86 ± 0·87 (A)	0·47 ± 0·35
T 1·19 ± 0·35	1·98 ± 0·92	1·47 ± 0·47 (B)	1·14 ± 0·84
L 0·55 ± 0·41	1·48 ± 0·25	0·55 ± 0·18 (C)	0·45 ± 0·20
3-day-old culture			
N 0·49 ± 0·22	1·17 ± 0·89	1·33 ± 0·85 (D)	1·09 ± 0·50
T 1·28 ± 0·45	2·30 ± 0·60	1·9 ± 0·80 (E)	1·50 ± 0·59
L 0·79 ± 0·35	0·93 ± 0·78	0·35 ± 0·13 (F)	0·31 ± 0·16
7-day-old culture			
N 0·40 ± 0·27	0·62 ± 0·48	1·07 ± 0·59 (G)	0·73 ± 0·26
T 0·94 ± 0·30	2·13 ± 0·60	1·2 ± 0·48 (H)	0·90 ± 0·59
L 0·30 ± 0·047	0·39 ± 0·09	0·20 ± 0·10 (I)	0·19 ± 0·05

Results expressed (nmol/hr/10^6 cells).

N = normal ($n = 13$); T = tuberculoid ($n = 13$); L-lepromatous ($n = 8$) values expressed as mean ± SD [n as in ( )].

A–B = $p < 0{\cdot}01$; A–C = p = NS*, B–C = $p < 0{\cdot}001$, E–F = $p < 0{\cdot}001$, E–H = $p < 0{\cdot}05$; H–I = $p < 0{\cdot}001$.

* NS = not significant.

differently. The O_2^- production tapered off in all groups as the culture aged at any of the concentration of tuftsin.

H_2O_2 RELEASE

The H_2O_2 production was also measured in these cultures as a function of culture age (1–7 day). 1-day-old normal monocytes produced a basal level of 17·75 ± 2·3 nmol/hr/10^6 cells. When these cells were stimulated with PMA (32 nM), the levels of H_2O_2 rose to 476·75 ± 36·7 nmol/hr/10^6 cells. Tuftsin (0·22 μM) stimulation caused a 3-fold increase in H_2O_2 production when compared to basal level of 1-day-old cultures. The basal levels of BT/TT and BL/LL Mϕ cultures on day 1 were almost the same (8·39 ± 4·9 nmol/hr/10^6 cells and 7·3 ± 1·5 nmol/hr/10^6 cells, respectively). PMA stimulation showed higher H_2O_2 release in both the cases, and tuftsin stimulation (0·22 μM) showed approximately a 5–6-fold increase in H_2O_2 production. At a higher concentration of tuftsin (0·88 μM), normal and BT/TT Mϕ of 1-day-old cultures showed an inhibitory effect, whereas the levels of H_2O_2 produced by BL/LL Mϕ were comparatively less than 0·22 μM tuftsin stimulation. On the whole, a 3-fold increase in H_2O_2 production was observed when compared to the basal levels.

The 3-day-old cultures of normal and BT/TT behaved in a similar manner in the production of H_2O_2 under optimal conditions, whereas BL/LL Mϕ behaved differently. The basal production of H_2O_2 in normal and BT/TT Mϕ was almost the same (24·25 ± 2·8 nmol/hr/10^6 cells and 25·3 ± 12·0 nmol/hr/10^6 cells, respectively) although there is a slight

increment when compared to 1-day-old cultures. The BL/LL Mϕ showed lower levels of H_2O_2 production in unstimulated cultures ($5{\cdot}34 \pm 0{\cdot}65$ nmol/hr/10^6 cells). PMA stimulation showed a 20-fold increase in H_2O_2 production in all the groups. Tuftsin (0·22 μM) showed a 3–4-fold increase in H_2O_2 production in normal and BT/TT while there was approximately a 6-fold increase in BL/LL Mϕ cultures. At higher concentration of tuftsin (0·88 μM), the normal and BT/TT Mϕ showed basal levels similar to unstimulated cultures, while BL/LL Mϕ showed a 4-fold increase compared to the unstimulated cultures.

The 7-day-old cultures showed a decline in H_2O_2 production in all 3 groups when compared to 3-day-old cultures of unstimulated Mϕ, and although there is a decrease in H_2O_2 production in the 7-day-cultures, the fold increase within the same group in 3-day- and 7-day-old cultures are comparable.

ADA ACTIVITY

In 1 day Mϕ of normal and BT/TT, after pulsing with tuftsin (0·22 μM), the ADA activity was almost the same (normal $22{\cdot}75 \pm 7{\cdot}02$ nmol/hr/10^6 cells; BT/TT $28{\cdot}39 \pm 7{\cdot}2$ nmol/hr/10^6 cells). The unstimulated cultures of normal and lepromatous types were unable to show detectable ADA activity, while the tuberculoid Mϕ did show this activity ($24{\cdot}37 \pm 1{\cdot}6$ nmol/hr/10^6 cells). Surprisingly, the lepromatous cultures showed higher ADA activity ($126{\cdot}12 \pm 33{\cdot}47$ nmol/hr/10^6 cells) on stimulation. At a higher concentration of tuftsin, the BT/TT and BL/LL Mϕ showed almost the same ADA activity. In 3-day-old normal cultures, tuftsin pulsing showed a 3-fold increase in ADA activity, while tuberculoid Mϕ failed to show a similar response. The BL/LL Mϕ also failed to show any detectable ADA activity in stimulated or unstimulated cultures. Higher tuftsin concentration (0·88 μM) showed less ADA activity than the unstimulated cultures.

In 7-day-old normal cultures there is a decrease in ADA activity in stimulated cells when compared to 3-day-old cultures. The unstimulated cultures failed to show any detectable ADA activity.

Discussion

Tuftsin is an endogenous immunomodulator for macrophage phagocytic and microbicidal functions.[13] One of the most important mechanisms of intracellular killing of ingested micro-organisms or pathogens by mononuclear cells is the generation of toxic oxygen products[1,14] through NADPH oxidase or xanthine oxidase pathways. The NADPH oxidase is dormant in non-activated phagocytes. It is activated when phagocytes are exposed to the appropriate stimuli and thereby contributes to the microbicidal activity of these cells. Infection leads to increased xanthine oxidase activity, thus also contributing to the release of toxic oxygen radicals. In this study we have compared the release of O_2^-, H_2O_2, and ADA activity in response to tuftsin pulsing on monocytes/macrophages derived from leprosy patients as a function of *in vitro* culture age. Our results show that 0·22 μM tuftsin is the optimal dose for *in vitro* stimulation, while higher doses of tuftsin (0·88 μM) show an inhibitory effect. This is in accordance with observations by others.[5,15]

The monocytes/macrophages were pulsed with tuftsin and the time kinetics were

studied at 30, 60 and 90 min to determine the optimal time for the maximal release of these metabolites. Maximal release was obtained at 60 min (data not shown). The present study clearly demonstrates that tuftsin augments the release of ROI even in the absence of a phagocytic event.[11,16,17,18] Moreover, the release of ROI was related to the age of monocyte derived macrophages as well as the clinical spectrum of the disease.

The basal values of O_2^- released by unstimulated macrophages of tuberculoid individuals was higher than that of lepromatous and healthy subjects. Though in general the 3-day-old macrophage cultures showed maximal levels, the age of the macrophages did not alter the pattern of release. Similarly, tuftsin also showed maximal effects by day 3 on tuberculoid macrophages. In contrast, lepromatous macrophages were not stimulated by tuftsin. Hydrogen peroxide release was also maximal on day 3 for tuberculoid and normal as compared with lepromatous Mϕ. At all time points, the latter showed the lowest levels of H_2O_2. It was interesting to discover that tuftsin significantly improved H_2O_2 release of the hitherto poor lepromatous Mϕ. Tuberculoid and normal Mϕ needed a 3-day period to reach maximal levels, while lepromatous Mϕ released maximal levels on day 1. Lepromatous macrophages produced 50% as much H_2O_2 as that of normal macrophages upon stimulation with PMA or tuftsin, supporting the observations of Nathan *et al.*[19] Thus this immunomodulator had a differential effect on ROI production as it was ineffective in improving O_2^- release from the lepromatous Mϕ. Interestingly, tuftsin in lower concentrations was a more effective stimulator than at higher concentrations.

Our results show a high level of H_2O_2 release by BL/LL cultures as compared to O_2^-; molecular O_2 is reduced to O_2^- within the monocytes/macrophages at a site accessible to SOD, so that H_2O_2 is more likely to be secreted than O_2^- throughout the spectrum. Similar observations or failure to get sufficient O_2^- release has been noticed by other workers using lepromatous macrophages.[20,21,22] Mature macrophages are known to be associated with high SOD activity. The high H_2O_2 levels with BL/LL Mϕ observed in our study further support the view that they appear to be fully differentiated or matured. The level of differentiation and age of *in vitro* culture can be correlated with the production of reactive oxygen intermediates and biochemical enzymes associated with monocytes/macrophages. Tuprin *et al.*[23] have shown that small monocytes produce increasing amounts of H_2O_2 with increasing culture age *in vitro*. In addition, these cells were stimulatory towards LPS and γ-IFN. However, large monocytes showed decreased H_2O_2 production with increasing age of culture, an observation similar to our findings. It was also observed that peripheral blood PMNs of lepromatous patients were able to reduce Nitroblue tetrazolium[24,25] with an enhanced endogenous SOD activity.[26] Further, since *M. leprae* contained SOD activity, it is probably able to protect itself from the effect of the superoxide radical by converting O_2^- to the less toxic H_2O_2.[27] Therefore, when lepromatous leprosy or its like occurs, the multiplication/killing of *M. leprae* within the Mϕ is inhibited by the interaction between H_2O_2 and O_2^- and this is important for the subsequent release of other toxic radicals like OH$\cdot$ and O_2^1.[3] All these results led us to speculate that despite H_2O_2 generation in BL/LL patients another important metabolite like O_2^- is also essential to effectively kill *M. leprae*. The unresponsiveness of Mϕ to modulation, and its failure to become sufficiently activated by tuftsin, as seen in present study, especially in BL/LL Mϕ, could result in growth or multiplication of *M. leprae* inside the macrophages which is one of the characteristic features of lepromatous leprosy.[28] It also seems that the T cell activation and specific lymphokine secretion as well

Table 2. Day and 1-dose response profile of monocytes/macrophages cultured *in vitro* (1 day–7 day) of normal and leprosy patients for production of hydrogen peroxide

Unstimulated	PMA (32 nM)	Tuftsin (0·22 μM)	Tuftsin (0·88 μM)
1-day-old culture			
N 17·75±2·3	476·75±36·7	43·08±13·2 (A)	3·19±0·14
T 8·39±4·9	297±166	42·28±11·3 (B)	2·2±0·3
L 7·3±1·5	228±51·5	44·68±9·7 (C)	25·68±8·6
3-day-old culture			
N 24·25±2·8	692·25±32	79·96±8·9 (D)	30·3±4·2
T 25·3±12	507±34·2	95±32·7 (E)	32·12±13·5
L 5·34±0·65	231±42·8	31·5±1·62 (F)	21·12±6·8
7-day-old culture			
N 7·32±2	307·75±37	30±8·5 (G)	12·79±5·7
T 8·3±1·6	249±50	35·4±5·4 (H)	20·5±5·0
L 2·8±1·7	155±11·7	19·9±5·59 (I)	22·6±2·3

Results expressed (nmol/hr/10^6 cells).
N = normal ($n=8$); T = tuberculoid ($n=8$); L-lepromatous ($n=8$) values expressed as mean ± SD [n as in ( )].
A–D = $p<0{\cdot}001$; B–E = $p-<0{\cdot}001$; C–F = $p<0{\cdot}001$; D–G = $p<0{\cdot}001$; E–H = $0<0{\cdot}001$; F–I = $p<0{\cdot}05$.

as the unresponsiveness of macrophages for activation could also be linked to aberrant Mϕ function in LL patients.

Fischer *et al.*[6] have shown the importance of ADA for normal macrophage function and for the maturation of monocytes to macrophages. A hereditary deficiency of ADA has been shown to be associated with defective cellular and humoral immunity.[29]

In our study, except in 1-day BL/LL macrophage, measurable ADA activity was not detected in 3-day- and 7-day-old cultures. Since no measurable ADA activity was seen after 60 min of tuftsin pulsing, a parallel 15-min experiment was conducted with tuftsin pulsing to discover if ADA activity was detectable any earlier, but measurable ADA activity was not detected in these cultures. Interestingly, we found a direct relationship between ADA activity and O_2^- generation in normal and BT/TT groups.

Our findings show that fresh monocytes (1 day old) from either end of the spectrum of leprosy have basal production of metabolites, and after pulsing with an optimal dose of tuftsin the levels of these metabolites increase progressively. This increase was statistically significant ($p<0{\cdot}01$) (Tables 1–3). The basal level of ROI production is consistent with our earlier findings that the rate of microbicidal activity by Mϕ from all 3 groups did not appear to be impaired.[8] The 3-day-old cultures of normal and BT/TT Mϕ behaved similarly in the absence of any stimulant, but on stimulation showed a progressive rise in ROI production, a finding similar to that seen with human monocytes when they differentiate to macrophages *in vitro*.[27] The macrophages of BL/LL patients behaved as if they had prematurely aged and differentiated. In all 3 groups, the 7-day-old cultures failed to generate ROI production.

Table 3. Day and dose response of monocytes/macrophages derived from peripheral blood of normal and leprosy patients for adenosine deaminase activity

Unstimulated	PMA (32 nM)	Tuftsin (0·22 μM)	Tuftsin (0·88 μM)
1-day-old culture			
N ND	44·12 ± 5·32	22·75 ± 7·02 (A)	14·0 ± 11·9
T 24·37 ± 1·6	19·65 ± 10·88	28·39 ± 7·2	38·17 ± 4·07
L ND	ND	126·12 ± 33·47	37·91 ± 22·5
3-day-old culture			
N 31·17 ± 1·05	54·00 ± 26·2	93·16 ± 31·9 (B)	22·7 ± 6·4
T 30·9 ± 6·7	37·3 ± 7·9	25·4 ± 9·2	15·5 ± 6·5
L ND	ND	ND	ND
7-day-old culture			
N ND	43·36 ± 22·0	49·40 ± 5·5 (C)	15·75 ± 6·2
T ND	ND	ND	ND
L ND	ND	ND	ND

Results expressed (nmol/hr/10^6 cells).
N = normal ($n = 6$); T = tuberculoid ($n = 6$); L-lepromatous ($n = 6$).
ND = not detectable.
Values expressed as mean ± SD [n as in ( )].
A–B = $p < 0{\cdot}001$; B–C = $p < 0{\cdot}01$.

The possible cause of decreased ROI production seen with older cultures could be due to several possibilities. These are (a) an increased activity of H_2O_2 scavengers after day 3 of culture,[27] (b) specific enzyme/enzymes defects associated with ROI production; and (c) the functional heterogeneity of monocytes/macrophages as suggested by Norris *et al.*[30] Therefore, the differentiation of monocytes to macrophages *in vitro* involves a gradual transition of morphologic, physiologic, and biochemical changes, and the survival of a given pathogen inside it may depend on the level of differentiation of the monocyte that it infects.

This is the first reported study measuring ROI intermediates and ADA activity from the monocytes/macrophages of leprosy patients after tuftsin pulsing as a function of the age of culture. It is tempting to speculate that in addition to the protective mechanisms of the pathogen, biochemical factors are also involved in the defective macrophage function of lepromatous patients. We are currently measuring the levels of different second messengers like $[Ca^{++}]_i$, cyclic nucleotides and tuftsin receptor sites/affinities on the monocytes/macrophages derived from the leprosy individuals in the presence and absence of tuftsin stimulation.

Acknowledgements

We are grateful to CSIR, New Delhi, India for financial assistance and fellowship to one of us (SK). We wish to give special thanks to Dr I. Nath for critical comments, suggestions and for reviewing the manuscript.

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Modulation des monocytes/macrophages dérivés du sang périphérique de malades lépreux en utilisant 'Tuftsin' pour produire des intermédiaires de l'oxygène activé

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Résumé Les cellules de phagocytes répondent à divers stimulants de la membrane en produisant des intermédiaires de l'oxygène activé (ROI), c'est-à-dire les métabolites O_2^-, H_2O_2 et OH·. L'activation de la membrane plasmatique est associée à l'opxydase NADPH génératrice de superoxyde, ce qui provoque la production de ces substances toxiques. La stimulation des phagocytes aboutit également à l'activation du catabolisme de la purine, qui dirige le métabolisme, par la voie xanthine-oxydase, vers la production de l'anion superoxyde. Nous avions observé auparavant que les macrophages BL/LL (Mø) perdaient prématurément leur faculté de subir la phagocytose et l'activité microbicide stimulées par la tuftsine. L'objet de cette étude était de mesurer les taux de ROI avec ou sans flux de 'tuftsine' en fonction de l'âge de la culture *in vitro* et aussi de rapporter ces taux à l'activité adénosine-déaminase (ADA). On sait que cette dernière contribue à la production de O_2^- etr joue un rôle dans la maturation du système monocyte/macrophage. Le comportement des monocytes/macrophages mormaux et tuberculoïdes était similaire en présence ou en l'absence de tuftsine, c'est-à-dire qu'il présentait un accroissement progressif de la producton de ROI jusqu'au jour 3 de culture, puis un ralentissement sur les cultures plus vieilles, au jour 7. Par contre, après le jour 1, les macrophages lépromateux n'étaient pas capables d'être induits par la tuftsine à produire ROI et à activer ADA. Ces résultats mettent en évidence une déficience de la fonction Mø chez les patients lépromateux envers le flux tuftsine, ce qui confirme nos observations précédentes. Ainsi les macrophages BL/LL se comportent comme s'ils étaient déja vieillis après un jour de culture *in vitro*, ce qui expliquerait leur impuissance à éradiquer efficacemnt *Mycobacterium leprae*.

Modulación de los monocitos/macrófagos de la sangre periférica de pacientes leprosos, usando 'Tuftsin' para la producción de productos intermedios de oxígeno reactivo

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Resumen Les céculas fagocítas responden a muchos diferentes estimulantes de membrana mediante la producción de productos intermedios de oxígeno reactivo (ROI), por ejemplo, los metabolitos O_2^-, H_2O_2 y OH·. La activación de la membrana plásmica está asociada con oxidasa NADPH que genera superóxido, causando de este modo la producción de estas especies tóxicas. La estimulación de células fagocíticas también resulta en la activación del catabolismo purínico que dirige el flujo metabólico a través de la oxidasa xantínica para producir el anión de superóxido. Hemos observado con anterioridad que los micrófagos BL/LL exhiben una inhabilidad prematura de fagocitosis estimulado por el 'Tuftsin', y de actividad microbicida. El estudio actual fue realizado para medir los niveles de ROI en la ausencia y presencia de pulsación de 'Tuftsin', en función de la edad del cultivo en vitro, y también correlacionar estos niveles con la actividad de deaminasa adenosínica (ADA). Se sabe que éste es un contribuidor a la generación de O_2^-, y también está implicada en la maduración del sistema monocito/macrófago. El comportamiento de monocitos/macrófagos normales y tuberculoides es más o menos idéntico, sea en la presencia o ausencia de 'Tuftsin', es decir, hubo un aumento progresivo de la producción de ROI hasta el día número 3, luego hubo una disminución gradual en los cultivos más viejos. Vuelta en cambio, después del día 1, no se podían estimular los macrófagos lepromatosos mediante 'Tuftsin' para que tengan actividad ROI y ADA. Estos resultados indican una función Mϕ defectuosa a la pulsación de 'Tuftsin' por parte de los pacientes lepromatosos, confirmando nuestras observaciones anteriores. Así, el Mϕ BL/LL se comportó como si se hubiera envejecido después de un día en un cultivo en vitro, lo cual puede explicar su letalidad ineficaz frente a *Mycobacterium leprae*.