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Altered Antigen-Presenting Capacity of Human Monocytes after Phagocytosis of Bacteria

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The antigen-presenting and accessory functions of monocytes were studied after phagocytosis of bacteria. Peripheral blood monocytes isolated from mononuclear cells by counterflow elutriation were incubated with suspensions of opsonized bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, or *Salmonella enteritidis*) under conditions in which at least 80% of the monocytes engulfed microorganisms. Either the cells were pulsed with antigen (purified derivative of tuberculin or tetanus toxoid) and used as antigen-presenting cells for autologous T lymphocytes or the accessory function of the cells was examined in pokeweed mitogen-activated cultures of T cells. It has been found that phagocytosis of bacteria by monocytes reduces their ability to trigger antigen- and mitogen-induced proliferation. The reduced proliferative response of T lymphocytes was not due to a change of the kinetics of the response or triggering of suppressor mechanisms. Furthermore, antigen processing was not affected much after phagocytosis of bacteria since antigen-pulsed and paraformaldehyde-fixed cells containing bacteria were comparable to control cells in their antigen-presenting capacity. This phenomenon was observed after phagocytosis of both living and dead bacteria and was not correlated to the viability of monocytes, which were more affected after phagocytosis of living bacteria than of dead ones. As a result of phagocytosis of bacteria, reduced expression of CD54, CD14, and HLA-DQ, variable reduction of HLA-DP, CD58, and CD64, and reduced viability of monocytes were observed. In conclusion, phagocytosis of bacteria by monocytes affects their antigen-presenting and accessory functions presumably because of changes in the expression of molecules essential for monocyte–T-cell interactions and reduction of their viability.

Monocytes/macrophages are involved in various host-bacterium interactions. They are effector cells in the efferent limb of the immune response when they phagocytose and kill pathogens but can also trigger an immune response after processing bacterial antigens and presenting them to T lymphocytes (3, 14, 18, 20). Although the monocyte functions mentioned above are well known, it is not clear whether the same cell can act simultaneously as effector and antigen presenting cell (APC) and, if so, whether antigen presentation under these conditions remains unaltered. Apart from theoretical aspects, the answer to this question may contribute to our better understanding of the antibacterial immune response and the influence that infection might have locally on immune cells.

Bacteria and their products are known to influence several functions of monocytes/macrophages. They may activate them and change their immunoregulatory (suppressive) and cytotoxic activities (4, 9, 15, 27, 28, 31). It has already been shown that activation of antigen-presenting macrophages may lead to antigen-dependent T-cell depletion (9, 25).

In this report, we address the question whether phagocytosis of microorganisms affects the ability of monocytes to function as APC.

MATERIALS AND METHODS

Bacterial strains. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella enteritidis* (clinical isolate) were grown for 18 h on sugar broth, washed twice with a large volume of saline, and opsonized (30 min, 37°C) in the presence of 5% fresh human serum (pooled fresh human serum stored in aliquots at −70°C). After the cells were washed again, their density was measured spectrophotometrically (540 nm) and the number of cells was calculated by using previously determined standard curves (based on CFU counts). Finally, bacteria were adjusted to a concentration of 10^9/ml in phosphate-buffered saline (PBS).

For analysis of phagocytosis by flow cytometry, bacteria were incubated before opsonization for 2 h at 37°C in PBS containing 0.1% fluorescein isothiocyanate (BHD Chemicals Ltd., Poole, England). After these cells were washed, they were opsonized as described above. In some experiments, to kill bacteria, the bacterial cells in suspension before opsonization were preincubated for 30 min in a water bath at 72°C.

Cell populations. Peripheral blood mononuclear cells were isolated by using a standard Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient from heparin- or EDTA-treated blood from healthy donors. Peripheral blood mononuclear cells were suspended in Hanks' balanced salt solution supplemented with 1% autologous plasma and subjected to countercurrent centrifugal elutriation (with a Beckman JE-6B-Elutriation System equipped with a 5-ml Sanderson separation chamber) to obtain lymphocytes and monocytes (26).

The separation was done at 10°C and at a constant rotor speed (3,500 rpm). A total of 10^8 peripheral blood mononuclear cells (PBMC; 100 ml, 2×10^6/ml) was loaded at a flow rate of 18.5 ml/min and subsequently eluted by gradually increasing the flow to 25 ml/min. Twenty cell fractions (50 ml each) were collected, including the last which was collected

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while the centrifuge was turned off. Monocyte enrichment was confirmed by nonspecific esterase staining (85 to 95% positive) and/or expression of CD14 antigen (80 to 90% LeuM3 positive). Monocytes were washed once with cold RPMI medium and, until used, kept in an ice bath at 5×10^6/ml in incomplete medium (RPMI 1640 supplemented with L-glutamine and 10% fetal calf serum; all reagents from GIBCO, Grand Island, N.Y.). Cells collected in fractions 5 to 10 (which contained 80 to 90% CD3-positive cells) were pooled together and rosetted with AET-pretreated sheep erythrocytes to isolate T cells (16). Rosetting (T) and nonrosetting (non-T) lymphocytes were separated by using a Ficoll-Paque density gradient. Cells recovered from the pellet by osmotic shock (T cells) were washed twice with Hanks' balanced salt solution and finally adjusted to a concentration of 10^6/ml in complete medium (i.e., incomplete medium to which antibiotics were added).

Phagocytosis of bacteria and antigen pulse. Monocyte suspensions (2×10^6 to 5×10^6/ml) were incubated (37°C) in heparinized glass tubes with bacterial suspensions. The monocyte-to-bacterium ratio was 1:100 unless otherwise stated. In parallel, monocytes without bacteria were also incubated. After 0.5 h of incubation, samples were washed twice at $100 \times g$ in ice-cold RPMI medium to separate monocytes from an excess of free bacteria and resuspended in complete medium. Thereafter, control monocytes and monocytes with engulfed bacteria were divided into two parts. One part was pulsed with antigen (purified protein derivative [PPD; Statenserum Institute, Copenhagen, Denmark; 100 μg/ml] or tetanus toxoid [TT; Behring, Marburg, Germany; 20 Lf/ml]). The second part of the monocytes was incubated without antigen. After 2 to 3 h of antigen pulse, the cells were washed and adjusted to a concentration of 10^6/ml in complete medium. In some experiments, antigen-pulsed monocytes were fixed with paraformaldehyde (PF) by the slightly modified method of Moreno and Lipsky (20). Briefly, after the cells were washed twice in PBS, they were incubated in 1% PF for 2 min at 37°C. Thereafter, the reaction was stopped by adding an excess of ice-cold 0.06% glycyl-glycine, and cells were washed twice again with PBS prior to being resuspended in complete medium. To prevent any possible leakage of PF, these cells were incubated for 1 h more at 37°C and washed before being used.

Cell cultures. A total of 10^5 T lymphocytes was cultured in complete medium in triplicate in 0.2-ml aliquots in flat-bottom 96-well plates (NUNCLON; Nunc, Roskilde, Denmark) with 1×10^4 to 3×10^4 control or antigen-pulsed monocytes as indicated. In some cases, antigen or polyclonal activator was added directly to culture wells (PPD, 25 μg/ml; pokeweed mitogen [PWM; GIBCO], 1:200). Cells were cultured for 6 days at 37°C under 5% CO_2 with a 6-h terminal pulse of [^{3}H]thymidine (1 μCi per well). In some experiments, 50-μl aliquots of culture medium were taken from each well after 72 to 96 h of culture for determination of gamma interferon (IFN-γ) concentrations.

Flow cytometry. To examine cell phenotype, samples (10^6) of control monocytes or monocytes containing bacteria were cultured in 1-ml aliquots in flat-bottom 24-well culture plates (NUNCLON). These cultures were harvested after 2, 12, or 24 h, and cells were labelled with a panel of monoclonal antibodies against CD14 molecule, HLA-DR, -DQ, and -DP (all from Becton Dickinson), CD11a, CD18 (both from DAKO, Glostrup, Denmark), CD54, CD58 (both from Immunotech, Marseilles, France), and CD64 (clone 22; Medarex Inc., West Lebanon, N.H.).

Isotype-specific controls (Becton Dickinson) were usually also included. All procedures were performed at 0°C, and cells were analyzed the same day with a FACScan or FACStar Plus flow cytometer (Becton Dickinson). During acquisition, the threshold was set to exclude any possible free bacteria; otherwise, cells were acquired and analyzed ungated. In some experiments, propidium iodide (20 μg/ml) was used during flow cytometry analysis to evaluate the viability of the cells and to exclude dead cells from analysis.

Cytokine measurements. The concentration of IFN-γ was measured by using an enzyme-linked immunosorbent assay kit (kindly provided by H. Gallati, Hoffmann-La Roche, Basel, Switzerland) as described previously (24).

RESULTS

Phagocytosis of bacteria by peripheral blood monocytes. Preliminary experiments were devised to establish conditions under which the majority of monocytes would contain bacteria. Purified monocytes were incubated for 0.5 h with serum-preopsonized bacterial suspensions of various concentrations, washed, and, after routine Giemsa staining, analyzed for the presence of intracellular bacteria. Alternatively, live bacteria were labelled with fluorescein isothiocyanate, and the proportion of cells containing bacteria was measured in a cytofluorograph. As expected, the proportion of monocytes with intracellular bacteria depended on the bacterium-to-monocyte ratio during phagocytosis and was usually more than 80% at a ratio of 100:1 (not shown). This bacterium/monocyte ratio was used throughout experiments unless stated otherwise.

T-cell proliferation in response to monocytes with ingested bacteria. It has been described that some bacteria, when added to a coculture of T cells and monocytes, can trigger [^{3}H]thymidine uptake comparable to that triggered by TT (18). Under our culture conditions, the T-cell proliferation in response to monocytes which ingested bacteria was much lower than that in response to antigen (Fig. 1a). [^{3}H]thymidine uptake in cocultures of T lymphocytes and 10 to 30% monocytes with ingested bacteria was comparable or sometimes two to five times higher than that in cultures to which control monocytes were added (not shown) and much lower than the T-cell response to PPD, TT, or mitogen (see below). It was concluded that T-lymphocyte responses to monocytes with engulfed bacteria, if present, would not interfere with the readout of PPD- or TT-driven proliferation.

Antigen-presenting capacity of monocytes after phagocytosis of bacteria. Monocytes which ingested bacteria and control cells were pulsed with PPD for 2 h. Thereafter, cells were washed free of antigen and added (10%) to a culture of autologous T lymphocytes. Cultures were harvested after different time periods to measure T-cell proliferation. As shown in Fig. 1b, monocytes after phagocytosis of bacteria were much less efficient as than control cells. This difference was not due to shifts of the kinetics which were similar in all cultures. In the experiment shown in Fig. 1, the antigen-presenting function of monocytes which ingested *P. aeruginosa* or *E. coli* was less affected than that of monocytes after ingestion of other bacteria. However, this observation was not reproducible, and no correlation could be found between the degree of the reduced antigen-presenting function and the bacterial species taken up by monocytes (not shown). The reduced antigen-presenting capacity of monocytes after phagocytosis of bacteria was also seen when cells were pulsed with TT (Fig. 2). In this experiment, different numbers of monocytes were added to cultured lymphocytes, and in addition to T-cell proliferation, the production of IFN-γ was also measured. As shown, both T-cell proliferation and IFN-γ secretion in response to TT were reduced if monocytes were first allowed to ingest bacteria.

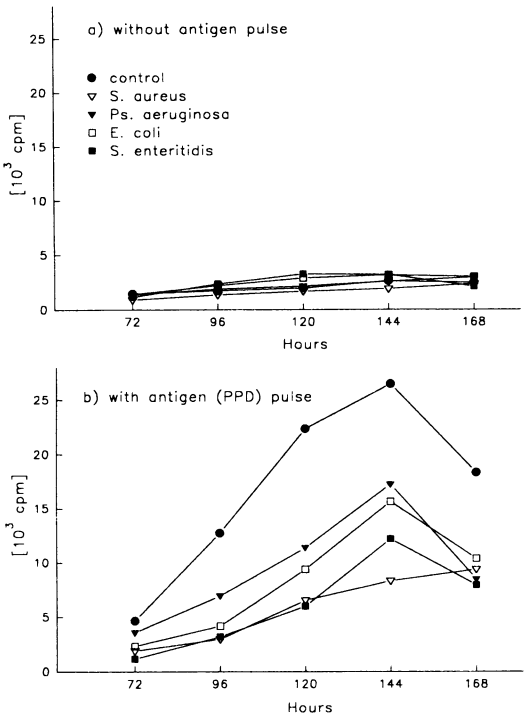

FIG. 1. T lymphocytes (10^5) were cultured in the presence of (10^4) free or bacterium-containing control (a) or PPD-pulsed (b) monocytes. Cultures were pulsed with [^{3}H]thymidine and harvested after the indicated time periods. The data are expressed as mean counts per minute of triplicate assays. The standard error was below 5% (not shown). Symbols shown on panel a apply to panel b as well.

Phagocytosis of bacteria by monocytes results in long-lasting impairment of their antigen-presenting and accessory functions. It was of interest to ask the question whether monocytes which ingest bacteria have only a temporarily reduced antigen-presenting and/or accessory capacity or whether this is a long-lasting phenomenon. This question was

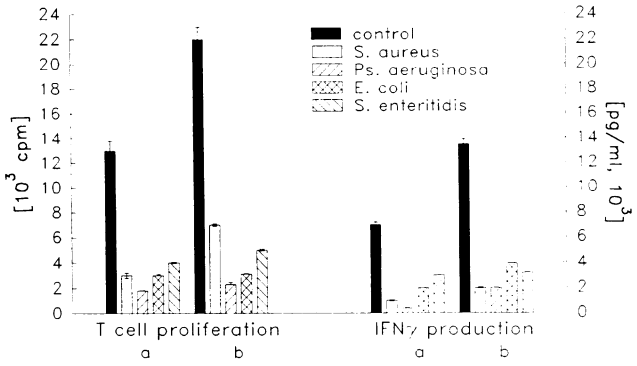

FIG. 2. T lymphocytes (10^5) were cultured in the presence of 10^4 (a) or 2×10^4 (b) TT-pulsed control or bacterium-containing monocytes. The results are shown as counts per minute (mean ± standard deviation) of T lymphocytes of triplicate determinations after 6 days and IFN-γ concentrations in culture supernatants after 3 days of culture. The T-cell response to unpulsed monocytes was negligible in the presence of both bacterium-containing and free monocytes (not shown).

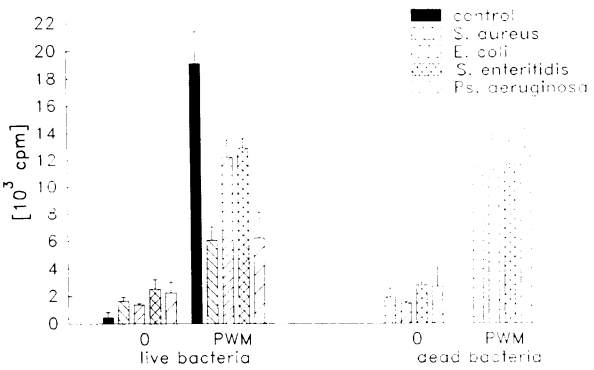

FIG. 3. T cells (10^5) were cultured in the presence of (10^4) control or bacterium-containing monocytes. To some of these, PWM was added and cells were cultured for 6 days before harvesting and measurement of [^{3}H]thymidine incorporation. The figure shows the results of one of six representative experiments in which the effects of phagocytosis of living and heat-killed bacteria were compared.

addressed in experiments in which monocytes were not pulsed but antigen or mitogen (i.e., PWM) was added to the culture medium. As shown in Fig. 3, monocytes which ingested bacteria could not support T-cell proliferation induced by the polyclonal activator PWM added to the culture. In addition, phagocytoses of living and dead bacteria had comparable effects on the ability of monocytes to support lectin-induced T-cell proliferation. Furthermore, when antigens (PPD or TT) were added to the cell cultures, T-cell proliferation was much lower when monocytes first engulfed viable or heat-killed bacteria (data not shown). In a separate experiment, IFN-γ production after PWM activation was also measured. The concentration of IFN-γ in culture with intact monocytes was 16.9 ng/ml, while in the presence of monocytes which ingested bacteria, it ranged from 2.2 (for *S. enteritidis*) to 5.8 (for *S. aureus*) ng/ml.

Phagocytosis of bacteria does not interfere with the ability of monocytes to process antigen. Antigen-specific T-cell proliferation and IFN-γ production require signals of at least two categories, i.e., signals derived through T-cell receptor (TCR) after recognition of the immunogenic peptide together with major histocompatibility complex class II antigens and accessory signals provided by cell-to-cell interactions (e.g., adhesion molecules) and cytokines (10, 30). In contrast, polyclonal, lectin-triggered T-cell proliferation requires only accessory signals. As monocytes containing bacteria were less effective than control cells in supporting lectin-triggered T-cell proliferation, it was of interest to see whether the reduced accessory function is the reason for their diminished antigen-presenting capacity. To answer this question, we took advantage of the fact that antigen processing and association with major histocompatibility complex class II can be dissected from the accessory function of APC (10, 20). Control and bacterium-containing monocytes were pulsed with TT and subsequently fixed with PF. These cells were used as APC alone or in combination with control intact monocytes as a source of accessory signals. As shown (Fig. 4), the treatment with PF completely abolished the antigen-presenting capacity of monocytes; however, the response could be restored to various degrees by adding unpulsed fresh monocytes as a source of accessory cells. In the control group, the restored response was always lower than it was prior to PF fixation. In contrast, the addition of accessory cells to cultures which had been activated by monocytes containing bacteria usually resulted in responses

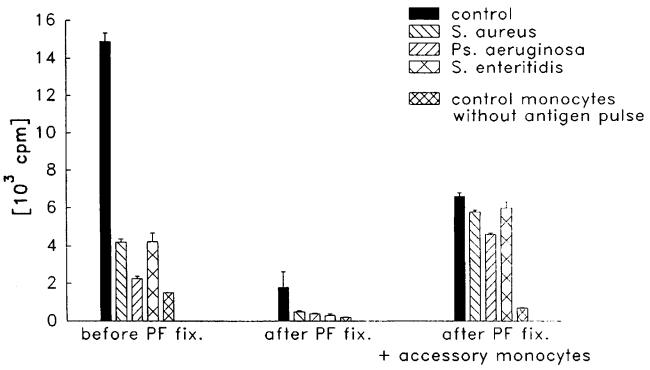

FIG. 4. Control or bacterium-containing monocytes were pulsed with TT and used (10^4 per well) as APC in a culture of autologous T lymphocytes (10^5 per well). These APC were used either intact (before PF fix.) or pretreated with PF (after PF fix.). In the case of the PF-pretreated cells, 5×10^3 of intact monocytes were also added to some cultures as indicated (+ accessory monocytes). The figure shows the proliferative response (mean ± standard deviation) of cultures done in quadruplicate. The response of T cells cultured in the presence of intact monocytes untreated or fixed with PF is also shown (control monocytes without antigen pulse).

higher than those before PF fixation. The supernatants of cultured (18 h) antigen-pulsed and PF-treated cells (0.5×10^6/ml) did not induced any significant T-cell proliferation when added (50%) to parallel cultures of incubated control T cells and monocytes (data not shown). This finding makes it unlikely that antigen or immunogenic peptides released by fixed cells were presented by control monocytes.

From these results, we conclude that the defective antigen-presenting function of monocytes which ingested bacteria is caused predominantly by an ineffective accessory function of these cells.

Monocytes with ingested bacteria do not induce suppressive circuits. Monocytes are known to possess immunoregulatory functions; namely, they can directly reduce lymphocyte response or trigger suppressive circuits (23). To determine whether any suppressive mechanisms are operating under our culture conditions, we compared the proliferative responses of T cells to a combination of antigen-pulsed control monocytes and monocytes containing bacteria. As shown in Table 1, monocytes which ingested bacteria and intact monocytes had similar effects on the T cell proliferation induced by antigen-pulsed control monocytes. Furthermore, increased numbers of antigen-pulsed monocytes induced higher T-cell responses independent of the presence or absence of bacteria inside the cells (see also Fig. 2). From these data, we conclude that monocytes containing bacteria do not differ in their immunoregulatory function from control cells.

Phenotype of monocytes after phagocytosis of bacteria. In some experiments, monocytes which have been used as APC or accessory cells were labelled with monoclonal antibodies against major histocompatibility complex class II antigens, adhesion molecules, FcRI (CD64), and CD14 monocyte differentiation antigen. The proportion of monocytes which expressed CD64, HLA-DR, CD11a, and CD18 was usually comparable to that of the control or, occasionally slightly lower. In contrast, the expression of HLA-DP, HLA-DQ, CD14, and CD54 was usually reduced (Table 2).

Phagocytosis of live or heat-killed bacteria resulted in comparable changes of monocyte surface antigens (data not shown). In some experiments, with the use of fluorescein isothiocyanate-labelled bacteria, the distinction was made between monocytes with and without bacteria to document that the observed changes are indeed associated with phagocytosis (Fig. 5). By this approach, we recently observed that, although down-regulation of the antigens mentioned above depends on the number of bacteria ingested by monocytes (a high bacterial load induces greater changes), the changes are not restricted to cells containing bacteria (1a).

TABLE 1. Effects of monocytes which ingested bacteria and intact monocytes on T-cell proliferation

Expt	Type (%)[a] of monocytes cultured with T lymphocytes	T-cell proliferation (10^3 cpm ± SD)
I	None	0.12 ± 0.01
	Intact (20)	0.35 ± 0.01
	PPD pulsed (10)	28.58 ± 1.12
	S. aureus[b] (20)	0.82 ± 0.09
	S. aureus PPD pulsed (10)	7.85 ± 0.32
	S. enteritidis (20)	0.54 ± 0.03
	S. enteritidis, PPD pulsed (10)	4.11 ± 0.09
	PPD pulsed (20)	47.27 ± 0.95
	S. aureus, PPD pulsed (20)	24.63 ± 2.10
	S. enteritidis, PPD pulsed (20)	14.30 ± 2.02
	PPD pulsed (10)	
	+Intact (10)	19.09 ± 1.98
	+*S. aureus* (10)	21.42 ± 2.20
	+*S. enteritidis* (10)	17.81 ± 1.04
	+*S. aureus*, PPD pulsed (10)	25.12 ± 3.11
	+*S. enteritidis*, PPD pulsed (10)	23.99 ± 1.09
II	TT pulsed (10)	20.08 ± 1.24
	+Intact (10)	17.80 ± 0.78
	+*S. aureus* (10)	23.57 ± 1.13
	+*S. enteritidis* (10)	14.58 ± 2.32

[a] Either 10% (10^4) or 20% (2×10^4) monocytes were added to 10^5 lymphocytes.
[b] Bacterial names indicate the type of bacteria ingested by the monocytes.

While performing flow cytometry analysis, we noted that some cells incubated with viable or heat-killed bacteria change their light-scattering properties in that they have reduced forward light scatter (FSC). The proportion of cells with reduced FSC ranged between 15 and 30% shortly after phagocytosis and was 25 to 50% after 24 h of incubation. In the population of control monocytes, the proportion of cells with reduced FSC was at the beginning below 10% and between 10 and 20% after 24 h of culture. When cells were analyzed in the presence of propidium iodide, it has been found that a variable proportion, i.e., 20 to 35%, of the cells, predominantly within the population with the reduced FSC, were propidium iodide positive. The reduction of cell viability was more pronounced when viable bacteria were incubated with monocytes and did not exceed 25% in the population of monocytes which phagocytosed heat-killed bacteria. No reproducible pattern of the reduction of FSC signal and viability was noted after phagocytosis of different strains of bacteria except that changes were always greatest after phagocytosis of *S. aureus* (on two occasions the viability in samples incubated with viable *S. aureus* was only 40%).

DISCUSSION

The aim of this study was to determine whether phagocytosis of bacteria by monocytes changes their ability to trigger antigen- and lectin-driven T-cell responses. Various products of extracellular bacteria, such as lipopolysaccharides, toxins, and the growing list of superantigens, have been shown to interact with monocytes and lymphocytes (2, 8, 12, 13, 19, 21,

TABLE 2. Phenotype of monocytes after phagocytosis of bacteria[a]

Type of monocytes (% of cells stained with PI)[b]	% Expression (mean fluorescence channel)					
	HLA-DR	HLA-DP	HLA-DQ	CD54	CD64	CD14
Control (2.5)						
A	94.8 (422)	62.0 (127)	34.2 (54)	71.9 (212)	82.1 (58)	84.3 (707)
B	94.5 (422)	61.2 (123)	35.8 (54)	72.3 (212)	82.5 (59)	84.6 (707)
S. aureus containing (39.3)						
A	75.3 (295)	38.0 (124)	11.9 (60)	45.3 (135)	29.8 (52)	60.9 (445)
B	81.0 (263)	28.1 (116)	12.3 (48)	36.8 (126)	25.8 (53)	58.7 (434)
E. coli containing (13.0)						
A	85.1 (317)	42.7 (111)	27.3 (62)	59.3 (204)	80.4 (54)	38.5 (276)
B	83.2 (306)	45.8 (98)	21.3 (53)	68.5 (182)	77.5 (55)	45.0 (316)
S. enteritidis containing (15.5)						
A	80.1 (389)	37.1 (187)	25.3 (59)	3.3 (182)	71.5 (59)	41.0 (322)
B	81.0 (370)	38.9 (193)	26.7 (62)	41.1 (154)	68.4 (62)	59.3 (351)
P. aeruginosa containing (23.4)						
A	88.5 (382)	54.5 (181)	30.0 (53)	68.5 (197)	85.0 (57)	72.5 (418)
B	92.7 (405)	51.8 (172)	29.2 (55)	62.8 (181)	80.5 (53)	70.0 (448)

[a] Cells were cultured for 18 h after phagocytosis of bacteria and later labelled with the indicated monoclonal antibodies. The table shows the immunophenotypes of cells which were used in the experiment illustrated by Fig. 2.
[b] Cells were analyzed before (A) and after (B) exclusion of dead PI-stained cells.

32). It is already known that intracellular bacteria like mycobacteria or their products are able to interfere with cell functions essential for antigen processing. They can disturb phagosome-lysosome fusion, alter lysosomal pH, and interfere with cell activation and expression of surface antigens (1, 4, 28, 29). In our studies, we have used extracellular bacteria as a phagocytic stimulus to determine whether phagocytosis would have any impact on monocyte performance as APC. With the experimental conditions we employed, these bacteria were present in 70 to 95% of cells when the bacteria were incubated with monocytes at a ratio of 100:1. Therefore, we believe that monocytes which have been used as APC or accessory cells contained bacteria.

We have shown that monocytes after ingestion of bacteria are defective as APC and that their costimulatory functions are changed. The antigen-driven T-cell proliferation and IFN-γ production were much lower when monocytes with bacteria were used as APC.

It has been reported that, in the mouse, activation of APC may result in antigen-specific T-cell killing (9, 25). Since phagocytosis of bacteria results in strong monocyte activation, one could argue that such mechanisms are operating in our experimental conditions. Although this possibility was not formally excluded, we think it is unlikely since monocytes containing bacteria did not interfere with the response triggered by control antigen-pulsed monocytes. Furthermore, monocytes which ingested bacteria were neither suppressive by themselves nor triggering suppressive circuits when added to PWM-activated cultures of T and B lymphocytes (24a).

PPD and TT, the antigens used in our studies, require processing by APC before they can be recognized by T lymphocytes (3, 20, 33). In addition, accessory, antigen-independent signals, which involve cell-cell interactions, e.g., through adhesion molecules and cytokines, are necessary for T-cell activation (reviewed in reference 10). By the use of PF fixation to dissect antigen-presenting and accessory functions, we were able to show that the reduced ability to trigger an antigen-specific response was due mainly to impairment of the latter. In line with this conclusion, we observed a reduced ability of monocytes containing bacteria to support lectin-triggered T-cell proliferation and IFN-γ production. Recently, Tsuyuguchi et al. (29) reported that monocytes treated with the *Mycobacterium avium-Mycobacterium intracellulare* complex exhibit a reduced expression of CD14 and CD11b, while expression of HLA-DR and production of IL-1 are not affected. In this regard, we also observed the reduction of CD14 and of ICAM (CD54), the ligand for LFA-1. This may at least partially explain why monocytes which have taken up bacteria are less efficient as APC. It is conceivable that some additional mechanisms may also operate in cultures to which monocytes with bacteria are added. One such mechanism may be related to the reduced viability of APC. The reduced viability of monocytes during the first 24 h of culture could not, however, solely account for the results we obtained since no correlation was found between the viability of bacteria containing monocytes and their ability to trigger antigen- or lectin-driven T-cell proliferation. Phagocytosis of different bacteria resulted in comparable reductions of their ability to assist an antigen- or lectin-induced T-cell response, although the viability of monocytes which ingested *S. aureus* was always the lowest. Furthermore, ingestion of heat-killed bacteria, although effectively reducing monocyte antigen-presenting function, had only a minor effect on their viability. Finally, the observed alterations of monocyte function could not be overcome by increased cell numbers added to the culture. Nevertheless, we do not dismiss the possibility that the reduced viability of monocytes might be responsible for the ineffective accessory function of monocytes containing bacteria. The induction of optimal T-cell responses requires prolonged signaling (i.e., lasting several hours) by APC and/or accessory cells (6). We and others have shown that APC and accessory cells are eliminated by activated T cells (7, 11, 24). In lectin-activated cultures, this takes 48 to 72 h, and an additional supply of accessory cells can increase the response and change its kinetics (22, 24). Therefore, one can anticipate that a fast elimination of APC would result in a lower response. In this regard, it is of interest that flow cytometry analysis of monocytes after ingestion of bacteria shows a reduction of the FSC signal and cell viability as evidenced by propidium iodide uptake. Although we have no formal proof, the reduction of the FSC signal is known to be associated with programmed cell death, i.e., apoptosis, a phenomenon also described in the case of APC elimination (5, 11, 17). This finding is presently under our investigation.

In conclusion, we suggest that phenotypic changes and the

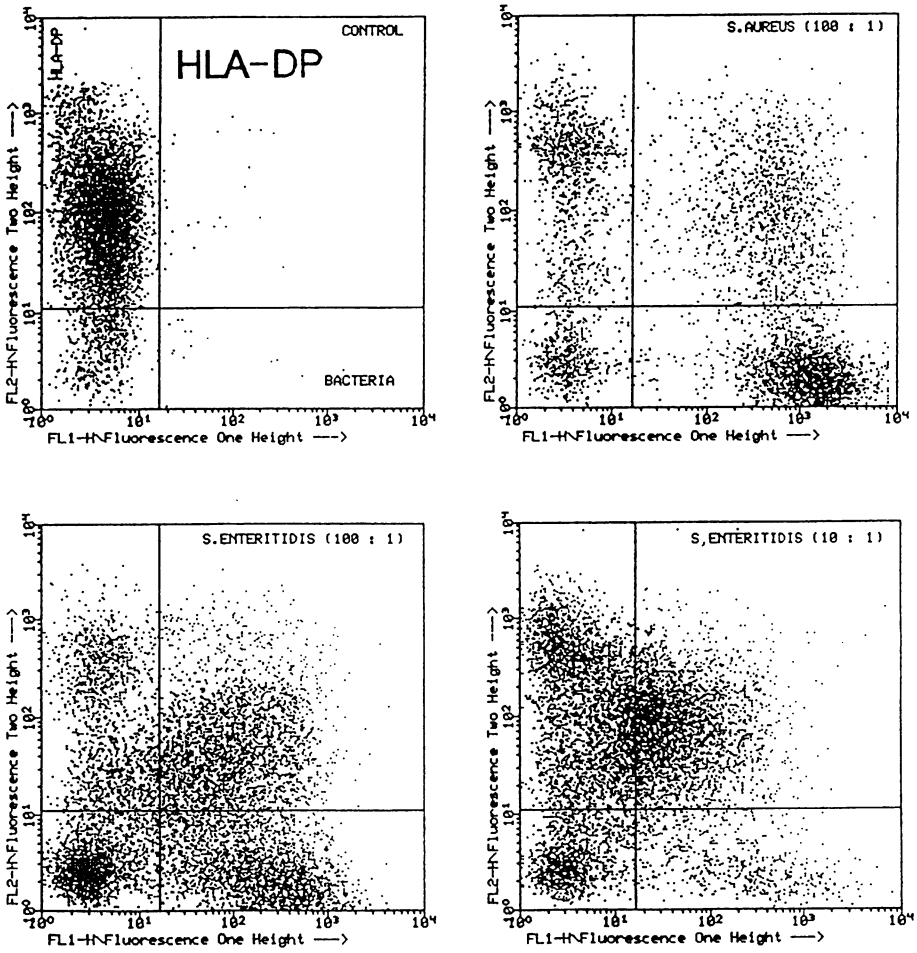

FIG. 5. Control or bacterium (FITC-labelled *S. aureus* or *S. enteritidis*)-containing monocytes cultured for 12 h were labelled with anti HLA-DP monoclonal antibody and goat anti-mouse immunoglobulin (phycoerythrin conjugated). Dot plots show green (FL1) versus red (FL2) fluorescence of control and bacterium-containing monocytes. The bacterium/monocyte ratio during phagocytosis is shown in parentheses after the name of the bacterium.

reduced viability (apoptosis) of monocytes/macrophages may be responsible for their reduced antigen-presenting capacity after phagocytosis of bacteria and represent a mechanism which in vivo prevents the immune system to respond to antigens which can easily be eliminated by other mechanisms.

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REFERENCES

1. **Armstrong, J. A., and D. Hart.** 1971. Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. J. Exp. Med. **134:**713–740.
1a. **Baran, J., and J. Pryjma.** Submitted for publication.
2. **Ben-Nun, A.** 1991. Staphylococcal enterotoxin B as a potent suppressant of T cell proliferative responses in rats. Eur. J. Immunol. **21:**815–818.
3. **Bhardwaj, V., and J. Colston.** 1988. The processing and presentation of mycobacterial antigens by human monocytes. Eur. J. Immunol. **18:**691–696.
4. **Chicurel, M., E. Garcia, and F. Goodsaid.** 1988. Modulation of macrophage lysosomal pH by *Mycobacterium tuberculosis*-derived proteins. Infect. Immun. **56:**479–483.
5. **Cohen, J. J.** 1991. Programmed cell death in the immune system. Adv. Immunol. **50:**55–85.
6. **Davis, L. S., and P. E. Lipsky.** 1989. T cell activation induced by anti-CD3 antibodies requires prolonged stimulation of protein kinase C. Cell. Immunol. **118:**208–221.
7. **Dohlsten, M., G. Hedlund, and T. Kalland.** 1991. Staphylococcal-enterotoxin-dependent cell-mediated cytotoxicity. Immunol. Today **12:**147–150.
8. **Fischer, H., M. Dohlsten, U. Andersson, G. Hedlund, P. Ericsson, J. Hansson, and H. O. Sjorgen.** 1990. Production of TNF-alpha and TNF-beta by staphylococcal enterotoxin A activated human T cells. J. Immunol. **144:**4663–4669.
9. **Friedman, A., and D. I. Beller.** 1987. Simultaneous expression of Ia and cytocidal activity by macrophages, and the consequences for antigen presentation. Immunology **61:**435–441.
10. **Geppert, T. D., L. S. Davis, H. Gur, M. C. Wacholtz, and P. E. Lipsky.** 1990. Accessory cell signals involved in T-cell activation. Immunol. Rev. **117:**5–65.
11. **Grogg, D., S. Hahn, and P. Erb.** 1992. CD4+ T cell-mediated killing of major histocompatibility complex class II-positive antigen-presenting cells (APC). III. CD4+ cytotoxic T cells induce apoptosis of APC. Eur. J. Immunol. **22:**267–272.
12. **Herman, A., J. W. Kappler, P. Marrack, and A. M. Pullen.** 1991. Superantigens: mechanism of T-cell stimulation and role in immune responses. Annu. Rev. Immunol. **9:**745–772.
13. **Herrmann, T., P. Romero, S. Sartoris, F. Paiola, R. S. Accolla,**

J. L. Maryanski, and H. R. MacDonald. 1991. Staphylococcal enterotoxin-dependent lysis of MHC class II negative target cells by cytolytic T lymphocytes. J. Immunol. **146:**2504–2512.
14. **Kaufmann, S. H. E.** 1988. Immunity against intracellular bacteria: biological effector functions and antigen specificity of T lymphocytes. Curr. Top. Microbiol. Immunol. **138:**141–176.
15. **Keller, R., R. Gehri, and R. Keist.** 1992. The interaction of macrophages and bacteria: *Escherichia coli* species, bacterial lipopolysaccharide, and lipid A differ in their ability to induce tumoricidal activity and the secretion of reactive nitrogen intermediates in macrophages. Cell. Immunol. **141:**47–58.
16. **Madsen, M., and H. E. Johnsen.** 1979. A methodological study of E-rosette formation using AET-treated sheep red blood cells. J. Immunol. Methods **27:**61–74.
17. **Mangan, D. F., G. R. Welch, and S. M. Wahl.** 1991. Lypopolysaccharide, tumor necrosis factor-alfa, and IL-1 beta prevent programmed cell death (apoptosis) in human peripheral blood monocytes. J. Immunol. **146:**1541–1546.
18. **Mannhalter, J. W., G. J. Zlabinger, R. Ahmad, and M. Eibl.** 1983. Human T cell proliferation in response to *E. coli* presented by autologous macrophages is antigen specific. Clin. Exp. Immunol. **53:**95–102.
19. **Misfeldt, M. L., P. K. Legaard, S. E. Howell, M. H. Fornella, and R. D. le Grand.** 1990. Induction of interleukin-1 from murine peritoneal macrophages by *Pseudomonas aeruginosa* exotoxin A. Infect. Immun. **58:**978–982.
20. **Moreno, J., and P. E. Lipsky.** 1986. Differential ability of fixed antigen-presenting cells to stimulate nominal antigen-reactive and alloreactive T4 lymphocytes. J. Immunol. **136:**3579–3587.
21. **Newcomb, J. R., J.-S. Lin, and T. J. Rogers.** 1990. Requirement for accessory cells in suppression of MOPC-315 IgA secretion by staphylococcal enterotoxin B-induced T-suppressor cells. Cell. Immunol. **129:**528–537.
22. **Pryjma, J., M. Ernst, R. Fetting, M. Woloszyn, M. Zembala, and H.-D. Flad.** 1991. The role of monocytes in the induction and regulation of IFN-gamma production by lectin-activated human T lymphocytes. Eur. Cytokine Net. **2:**273–279.
23. **Pryjma, J., W. Uracz, and M. Zembala.** 1989. Immunoregulatory function of monocytes, p. 327–336. *In* M. Zembala and G. L. Asherson (ed.), Human monocytes, Academic Press Ltd., London.
24. **Pryjma, J., M. Zembala, A. Pituch-Noworolska, M. Ernst, J. van der Bosch, and H.-D. Flad.** 1990. Monocyte-T-cell interactions in pokeweed mitogen-activated cultures. Immunology **71:**397–403.
24a. **Pryjma, J., et al.** Unpublished data.
25. **Rao, A., S. J. Fass, L. J. Miller, P. S. Riback, and H. Cantor.** 1983. Lysis of inducer T cell clones by activated macrophages and macrophage-like cell lines. J. Exp. Med. **158:**1243.
26. **Sanderson, R. J., F. T. Shepperdson, A. E. Vatter, and D. W. Talmage.** 1977. Isolation and enumeration of peripheral blood monocytes. J. Immunol. **118:**1409–1414.
27. **Shenker, B. J., L. Vitale, and J. Slots.** 1991. Immunosuppressive effects of *Prevotella intermedia* on in vitro human lymphocyte activation. Infect. Immun. **59:**4583–4589.
28. **Sibley, L. D., S. W. Hunter, P. J. Brennan, and J. L. Krahenbuhl.** 1988. Mycobacterial lipoarabinomannan inhibits gamma interferon-mediated activation of macrophages. Infect. Immun. **56:**1232–1236.
29. **Tsuyuguchi, I., H. Kawasumi, T. Takashima, T. Tsuyuguchi, and S. Kashimoto.** 1990. *Mycobacterium avium-Mycobacterium intracellulare* complex-induced suppression of T-cell proliferation in vitro by regulation of monocyte accessory cell activity. Infect. Immun. **58:**1369–1378.
30. **Van Seventer, G. A., Y. Shimuzu, K. J. Horgan, G. E. G. Luce, D. Webb, and S. Shaw.** 1991. Remote T-cell co-stimulation via LFA-1/ICAM-1 and CD2/LFA-3: demonstration with immobilized ligand/mAb and implication in monocyte-mediated co-stimulation. Eur. J. Immunol. **21:**1711–1718.
31. **Weir, D. M., and C. C. Blackwell.** 1983. Interaction of bacteria with the immune system. J. Clin. Lab. Immunol. **10:**1–12.
32. **Wells, A. F., J. A. Hightower, C. Parks, E. Kufoy, and A. Fox.** 1989. Systemic injection of group A streptococcal peptydoglycan-polisaccharide complexes elicits persistent neutrophilia and monocytosis associated with polyarthritis in rats. Infect. Immun. **57:**351–358.
33. **Ziegler, H. K., C. A. Orlin, and C. W. Cluff.** 1987. Differential requirements for the processing and presentation of soluble and particulate bacterial antigens by macrophages. Eur. J. Immunol. **17:**1287–1296.