INFECTION AND IMMUNITY, May 1997, p. 1863–1869 Vol. 65, No. 5
0019-9567/97/$04.00+0

An In Vitro-Differentiated Human Cell Line as a Model System To Study the Interaction of *Neisseria gonorrhoeae* with Phagocytic Cells

CHRISTOF R. HAUCK,[1] DIRK LORENZEN,[2] JOACHIM SAAS,[1]† AND THOMAS F. MEYER[1,2]*

Abteilung Infektionsbiologie, Max-Planck-Institut für Biologie, D-72076 Tübingen,[1] and Max-Planck-Institut für Infektionsbiologie, D-10117 Berlin,[2] Germany

Received 13 December 1996/Returned for modification 13 January 1997/Accepted 24 February 1997

The extreme host specificity of pathogenic neisseriae limits investigations aimed at the analysis of bacterial-host interactions almost completely to the use of in vitro models. Although permanent epithelial and endothelial cell lines are already indispensable tools with respect to initial infection processes, studies concerning the interaction of neisseriae with phagocytic cells have been confined to primary human blood cells. We investigated the use of human leukemia-derived monocytic and myelomonocytic cell lines that can be differentiated in vitro towards phagocytic cells by a panel of chemical and biological reagents including cytokines, vitamin analogs, and antileukemia drugs. Whereas tumor necrosis factor alpha, gamma interferon, bufalin, or granulocyte-macrophage colony-stimulating factor only marginally increased the ability of monocytic MonoMac-6 and myelomonocytic JOSK-M cells to interact with the bacteria, retinoic acid and vitamin D_3 treatment for 2 to 4 days led to highly phagocytic cells that internalized gonococci in an Opa protein-specific manner. This is comparable to the phagocytosis by primary monocytes from human blood, where more than 80% of cells are infected with intracellular bacteria. The increased phagocytic activity of JOSK-M cells following in vitro differentiation was paralleled by enhanced oxidative burst capacity. Whereas undifferentiated cells responded to neither phorbol 12-myristate 13-acetate nor other known soluble and particulate stimuli, cells incubated with retinoic acid and bufalin showed the same pattern and the same intensity of oxidative burst activity in response to *Neisseria gonorrhoeae* as primary cells: Opa-expressing gonococci elicited an oxidative burst, whereas Opa$^-$ gonococci did not. The surface expression of major histocompatibility complex (MHC) class II molecules was only slightly changed after retinoic acid treatment. Also, phagocytosis of gonococci had no influence on MHC class II surface expression. Taken together, our results demonstrate that in vitro-differentiated human myelomonocytic JOSK-M cells provide a suitable model for the study of a variety of aspects of the gonococcal interaction with phagocytes.

The astounding antigenic variability of *Neisseria gonorrhoeae* is a paradigm for the immune escape strategy used by an extremely host-adapted pathogen. Even though these bacteria possess certain conservative surface structures, one key mechanism by which they evade the immune surveillance of the host is antigenic variation of, e.g., pilin, lipooligosaccharide, and Opa proteins (24, 25, 33). In addition, distinct gonococcal strains evoke different clinical pictures of the disease and the outcome of the infection varies from person to person.

It is estimated that more than half of infected females become asymptomatic carriers, whereas in the other portion the immune system reacts with a strong inflammatory response (14). In acute cases, neutrophils, abundant constituents of the purulent exudate released from the urethra or cervix, and monocytes are believed to build up a major first line of defense against gonococci (for reviews, see references 32 and 35). In addition, antigen-presenting phagocytes link the unspecific defense with the antigen-specific response system, which may either promote or protect from disease (3).

Despite the broad interest in the interaction of gonococci with phagocytic cells, the fate of gonococci after internalization, pathogen accommodation inside cells, and bacterial factors responsible for the outcome of this interaction are matters of debate (1, 7, 9, 10, 26, 41). Further questions regarding the influence of pathogenic neisseriae on the activation, antigen-processing, and antigen-presenting functions of phagocytic cells have not even been addressed. This might be due in part to the fact that a permanent cell line model for the study of the interaction of gonococci with phagocytic cells is not available and experiments have relied solely on freshly isolated human blood cells. Previous attempts to replace this highly variable and therefore not standardizable material with permanent cell lines have been unsuccessful (9).

Since most phagocytic cell lines are derived from immature precursor cells of monocytes and neutrophils, we tried to improve their phagocyte-specific performance with respect to gonococci by in vitro differentiation with a panel of inducing substances. We demonstrate here that myelomonocytic and monocytic cell lines can be caused by in vitro treatment not only to display a more differentiated phenotype but also to increase their phagocytic potential towards *N. gonorrhoeae*. The use of this novel cell line model for the phagocyte-gonococcus interaction should allow detailed investigations of the delicate balance between these pathogens and the phagocytic cells of the host.

* Corresponding author. Mailing address: Max-Planck-Institut für Biologie, Abteilung Infektionsbiologie, Spemannstr. 34, D-72076 Tübingen, Germany. Phone: 49-(0)7071-601222. Fax: 49-(0)7071-610379. E-mail: sinfbio@mpib-tuebingen.mpg.de.

† Present address: Max-Planck-Institut für Biochemie, 82152 Martinsried, Germany.

MATERIALS AND METHODS

Cells and culture. The human monocytic cell lines MonoMac-6 (47) and THP-1 (38), the myelomonocytic cell line JOSK-M (29), obtained from the

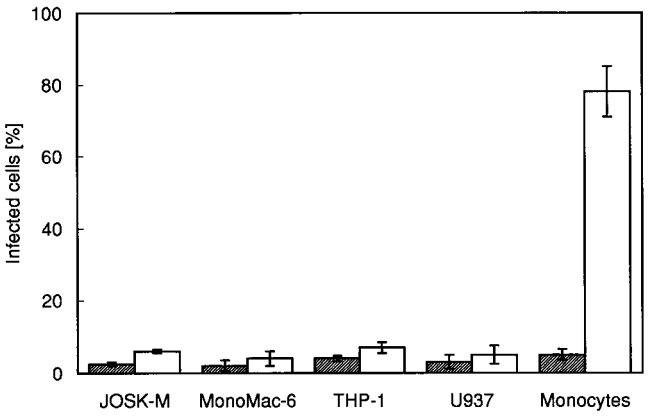

FIG. 1. Phagocytosis of *N. gonorrhoeae* MS11 by human histiocytic (U937), myelomonocytic (JOSK-M), and monocytic (THP-1 and MonoMac-6) cell lines and human primary monocytes in the absence of opsonins. Cells were incubated with 30 bacteria per cell for 4 h, and the percentage of cells containing phagocytosed gonococci was determined after Wright staining of the fixed preparations. Cells were incubated with Opa^- (hatched bars) or Opa_{50}^+ *N. gonorrhoeae* MS11 (open bars). The bars represent mean values ± standard deviations of three independent experiments.

German Collection of Microorganisms, Braunschweig, Germany (DSM ACC124, DSM ACC16, and DSM ACC30, respectively), and the human histiocytic line U937 (36) were grown as suspensions in RPMI 1640 (Gibco BRL, Paisley, Scotland) supplemented with 10% fetal calf serum (FCS; Boehringer GmbH, Mannheim, Germany) and 2 mM L-glutamine at 37°C in 5% CO_2. Cells were subcultured every 3 to 4 days.

Peripheral blood mononuclear cells were isolated from buffy coats of healthy adult donors by Ficoll Isopaque (1.077 g/ml; Biochrom) density gradient centrifugation (400 × *g*, 35 min, 22°C), washed twice with phosphate-buffered saline (PBS), layered on a Percoll gradient (1.068 g/ml; Pharmacia), and centrifuged at 400 × *g* in a fixed-angle rotor for 30 min at 22°C. Cells from the top of the gradient were collected and washed twice in PBS. The monocyte-enriched fractions contained approximately 60 to 80% monocytes as determined by differential blood cell counting with a Sysmex F-800 Microcellcounter (Digitana) or by α-naphthyl acetate esterase staining (Sigma). These cell suspensions contained more than 99% viable cells as assessed by the trypan blue exclusion test.

Bacteria. All of the gonococcal strains used in this work were derived from *N. gonorrhoeae* MS11 (23); variant F3 was the progenitor of the piliated strains, and variant B2 was the progenitor of the nonpiliated strains (20). The construction of *N. gonorrhoeae* MS11 strains which constitutively express distinct Opa proteins has been previously described (16, 17). All gonococci were grown on GC-agar (Gibco BRL) supplemented with vitamins and corresponding antibiotics at 37°C in 5% CO_2 and subcultured daily. Commensal neisseriae, obtained from U. Berger, Heidelberg, Germany, were grown on GC-agar and subcultured daily.

Differentiation of cell lines. Differentiation of cells was initiated by adding recombinant human gamma interferon (IFN-γ), recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF; Boehringer), vitamin D_3, recombinant human tumor necrosis factor alpha (TNF-α; Biomol), bufalin, retinoic acid, phorbol 12-myristate 13-acetate (PMA; Sigma), or combinations of these substances to exponentially growing cultures with a density of 5×10^5 cells/ml. Cells were incubated for 2 to 6 days, and viability was determined prior to infection by trypan blue staining and in all cases was >90%.

Infection with gonococci. For infection experiments, bacteria were taken from GC-agar plates, suspended in PBS, and washed by centrifugation at 1,000 × *g* in a microcentrifuge for 5 min. The optical density at 550 nm was determined in a DR 2000 spectrophotometer (Hach), and 3×10^7 bacteria were added to 10^6 JOSK-M, MonoMac-6, THP-1, or U937 cells suspended in RPMI 1640 containing 5% heat-inactivated FCS in 24-well plates (Nunc) to start the infection. After 4 h, uningested bacteria were separated from cells by repeated centrifugation at 120 × *g* in a microcentrifuge for 5 min and washing with PBS. Cells were spun down in PBS–20% FCS on microscope slides at 450 rpm for 6 min with a Cytospin 3 centrifuge (Shandon). Cytospin preparations were stained with Wright-Giemsa stain and documented with a Nikon FX-35A camera mounted on a Nikon Microphot-FX microscope and loaded with Kodak T-Max-400 or Ektachrome 160T film.

Measurement of the oxidative burst. To measure luminol-dependent chemiluminescence, JOSK-M or mononuclear cells were washed twice with PBS and suspended at 2×10^6/ml in PBS supplemented with 5 mM glucose and 0.2% bovine serum albumin (BSA) (CL-PBS). Luminol (dissolved in dimethyl sulfoxide; final concentration, 0.1 mM) was added to a 200-μl cell suspension, and the sample was incubated for 10 min at 37°C. The oxidative burst was initiated by adding PMA (dissolved in ethanol; final concentration, 0.1 μg/ml), ethanol (negative control; final concentration, 0.1%), or bacteria in PBS plus 5 mM glucose and 0.2% BSA at a ratio of 10 bacteria/cell. Chemiluminescence was recorded with six samples in parallel on a Multi-Bioluminat LB 9505C (Berthold) at 37°C for 60 min.

To check the percentage of cells that were able to generate superoxide anions, 2×10^5 differentiated and undifferentiated JOSK-M cells/ml were suspended in RPMI containing 10% FCS and 0.25% nitroblue tetrazolium salt (NBT; Serva). PMA was added to a final concentration of 2 μg/ml, and the cells were incubated for 25 min at 37°C. Cells were mounted on slides, counterstained with Wright stain, and monitored for NBT reduction.

Immunofluorescence staining and fluorescence-activated cell sorter analysis. Cells were washed twice in PBS at 4°C. Cells (10^5) were then incubated for 30 min on ice with anti-HLA-DR monoclonal antibody L243 (18) diluted in PBS containing 0.2% BSA. After two washes with PBS–0.2% BSA, cells were incubated with dichlorotriacyl-aminofluorescein-conjugated goat anti-mouse $F(ab')_2$ fragments (Dianova) in PBS–0.2% BSA for 30 min on ice. After another three washes with PBS, cells were fixed for 15 min at room temperature with 1% paraformaldehyde in PBS and then washed twice with PBS. Samples were analyzed in a FACSort analyzer (Becton-Dickinson) by gating viable monocytes according to their forward and sideward scatter and recording the fluorescence intensity (excitation at 488 nm, emission at 530 nm) of 10^4 cells.

RESULTS

Infection of different phagocytic cell lines with regard to their differentiation state. In a first trial, we tested the phagocytic activities of undifferentiated myelomonocytic (JOSK-M), histiocytic (U937), and monocytic (MonoMac-6 and THP-1) human cell lines against unopsonized, nonpiliated *N. gonorrhoeae*. Compared to primary monocytes obtained from human peripheral blood, these undifferentiated cell lines were unable to internalize gonococci whether or not the bacteria expressed Opa proteins (Fig. 1). Since in the absence of pili the interaction of unopsonized gonococci with primary monocytes is dependent on Opa proteins (Fig. 1) (8, 25, 42), Opa-expressing *N. gonorrhoeae* MS11 cells were used in the following experiments unless otherwise stated (16).

To improve their phagocytic performance, JOSK-M and MonoMac-6 cells were cultured in the presence of differentiation-inducing substances for 2 to 4 days (Table 1). Although MonoMac-6 and JOSK-M cells interacted to different degrees with gonococci following stimulation, their responses to the agents used showed similar patterns. Incubation of either cell line with TNF-α at 2 ng/ml, bufalin at 10 nM, or GM-CSF at 1 ng/ml was not sufficient to substantially alter the infection rate (less than 9% of the cells contained gonococci). Also, IFN-γ only slightly improved the ability of JOSK-M and MonoMac-6

TABLE 1. Infection of human phagocytic cell lines with *N. gonorrhoeae* MS11[a]

Reagent(s)	Concn(s)	Treatment duration (days)	Mean % of cells infected ± SD		
			JOSK-M	MonoMac-6	Primary monocytes
None			5.8 ± 1.2	3.8 ± 2.3	78.3 ± 7.2
Bufalin	10 nM	4	3.2 ± 1.0	1.8 ± 1.8	
IFN-γ	500 U/ml	3	23.1 ± 1.4	12.1 ± 6.9	
GM-CSF	1 ng/ml	3	8.1 ± 0.9	8.7 ± 4.5	
PMA	10 ng/ml	2	38.3 ± 2.5	21.3 ± 3.7	
RA[b]	100 nM	4	55.8 ± 3.9	17.1 ± 1.0	
TNF-α	2 ng/ml	3	5.8 ± 0.8	6.6 ± 0.6	
Vitamin D_3	150 nM	4	53.1 ± 4.2	15.8 ± 5.4	
RA, bufalin	100, 10 nM	4	70.4 ± 9.3	14.2 ± 7.3	

[a] Cells were incubated with differentiation-inducing agents for the times indicated, infected with nonpiliated, Opa^+ gonococci, incubated for 4 h, and stained as described in Materials and Methods. At least 200 cells were counted per treatment, and the percentage of infected cells was determined in three to five independent experiments.

[b] RA, retinoic acid.

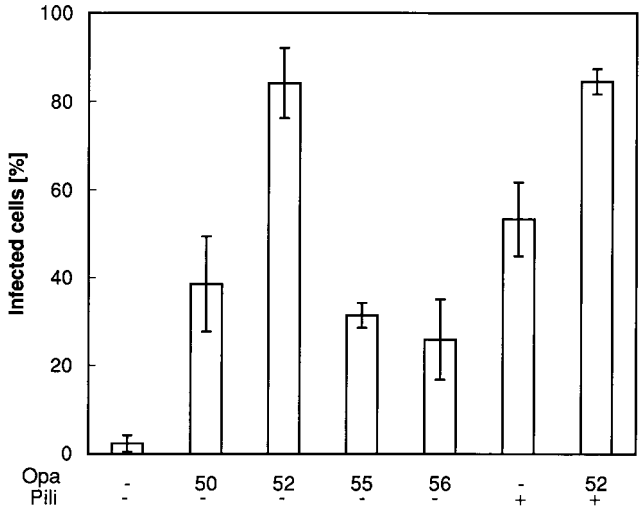

FIG. 2. Phagocytosis of *N. gonorrhoeae* MS11 expressing different Opa proteins and/or pili by JOSK-M cells differentiated with retinoic acid and bufalin. Cells were differentiated for 6 days and incubated with 30 bacteria per cell for 4 h, and the percentage of cells containing phagocytosed gonococci was determined after Wright staining of the fixed preparations. The bars represent mean values ± standard deviations of at least three independent experiments.

cells to phagocytose the bacteria. In contrast, PMA (10 ng/ml), vitamin D_3 (150 nM), or retinoic acid (200 nM) treatment led to a marked increase in the number of cells internalizing *N. gonorrhoeae* (Table 1). The two most active compounds (vitamin D_3 and retinoic acid) had clear dose- and time-dependent effects on the phagocytic activity of JOSK-M and MonoMac-6 cells (data not shown). To investigate if the results obtained by treatment with single substances could be potentiated, JOSK-M cells were treated with combinations of differentiation-inducing agents by using several different concentrations and time regimens. Whereas most combinations yielded only a marginal increase in the infection rate (data not shown), treatment with 100 nM retinoic acid and 10 nM bufalin for 4 days led to highly phagocytic cells. These in vitro-differentiated JOSK-M cells internalized *N. gonorrhoeae* to an extent comparable to that of primary monocytes in a highly reproducible manner (Table 1).

Interaction of different Opa-expressing and piliated *N. gonorrhoeae* strains with differentiated JOSK-M cells. To validate the cell culture model, retinoic acid-bufalin-treated JOSK-M cells were exposed after 6 days of differentiation to different opaque and/or piliated variants of *N. gonorrhoeae* MS11. As is known from earlier investigations (1, 10, 16, 28), primary human phagocytic cells interact with nonpiliated gonococci in an Opa protein-dependent fashion, with the different Opa protein variants expressed by the bacteria determining the degree of interaction with the phagocytes. As was shown for human primary blood cells (16), differentiated JOSK-M cells took up gonococci depending on the expression of Opa proteins: Opa^- gonococci were internalized by less than 3% of the cells, whereas bacteria expressing Opa_{52} were phagocytosed very efficiently (81%; Fig. 2 and 3). *N. gonorrhoeae* bacteria expressing Opa_{50}, Opa_{55}, or Opa_{56}, respectively, were also internalized by JOSK-M cells but to a significantly lower degree than Opa_{52}-expressing gonococci, infecting 39, 32, and 26% of the cells, respectively (Fig. 2). JOSK-M cells also internalized piliated, Opa^- neisseriae (53% infected cells), but phagocytosis was strongly improved if the gonococci expressed Opa_{52} in addition to pili.

Irrespective of piliation or the expressed Opa protein, internalized bacteria were always found to reside in membrane-bound compartments of the phagocytic cells. In most cases, these vesicles contained single diplococci, but especially Opa_{52}-expressing bacteria were often found in high numbers within large vacuoles (Fig. 3B). After infection with Opa_{52}-expressing gonococci, between 1 and 20 diplococci could be detected in about 15% of the infected cells, whereas about 85% of the infected cells contained more than 20 diplococci.

Differentiation by retinoic acid-bufalin treatment enables JOSK-M cells to generate an oxidative burst. Since differentiation influences many functions of the cell, we investigated if the change in phagocytic activity of JOSK-M cells was paralleled by alterations in other cell type-specific features. The ability to generate reactive oxygen derivatives in response to a panel of stimuli, the so-called respiratory or oxidative burst, is a well-known characteristic of mature phagocytes. Measurement of luminol-enhanced chemiluminescence showed that undifferentiated JOSK-M cells were unable to generate an oxidative response when stimulated with PMA (Fig. 4A). In contrast, already 2 days after the addition of retinoic acid and bufalin to the cell culture, PMA stimulation elicited detectable respiratory burst activity in JOSK-M cells. On day 5 after the beginning of the retinoic acid-bufalin treatment, PMA stimu-

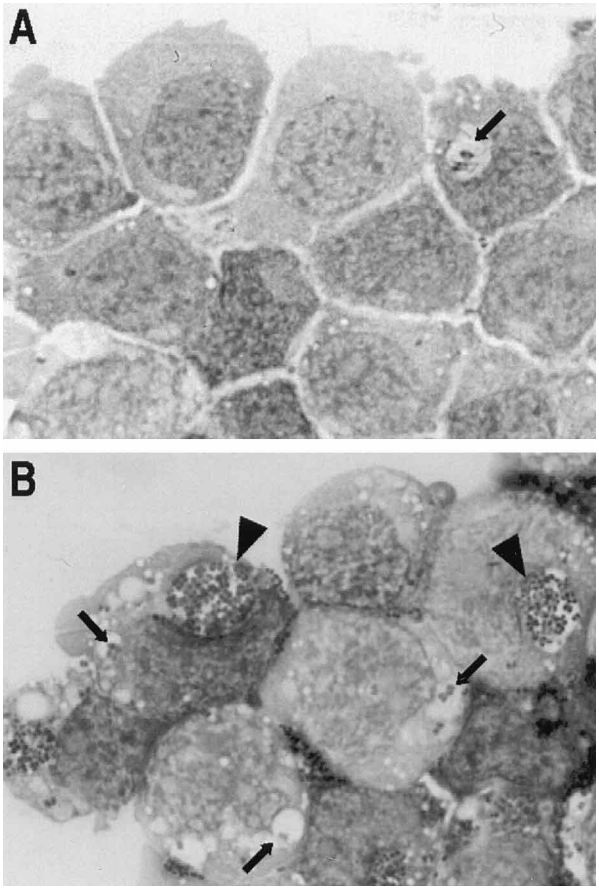

FIG. 3. Internalization of Opa^+ *N. gonorrhoeae* MS11 by JOSK-M cells. After Wright-Giemsa staining of infected cultures, gonococci can be clearly detected as dark diplococci within vacuoles of infected cells. Whereas JOSK-M cells only rarely take up Opa^- bacteria (A, arrow), Opa_{52}-expressing *N. gonorrhoeae* organisms are found in most of the cells (B, arrows). In many infected cells, Opa_{52}-expressing bacteria can be seen within large vacuoles containing more than 20 diplococci (B, arrowheads).

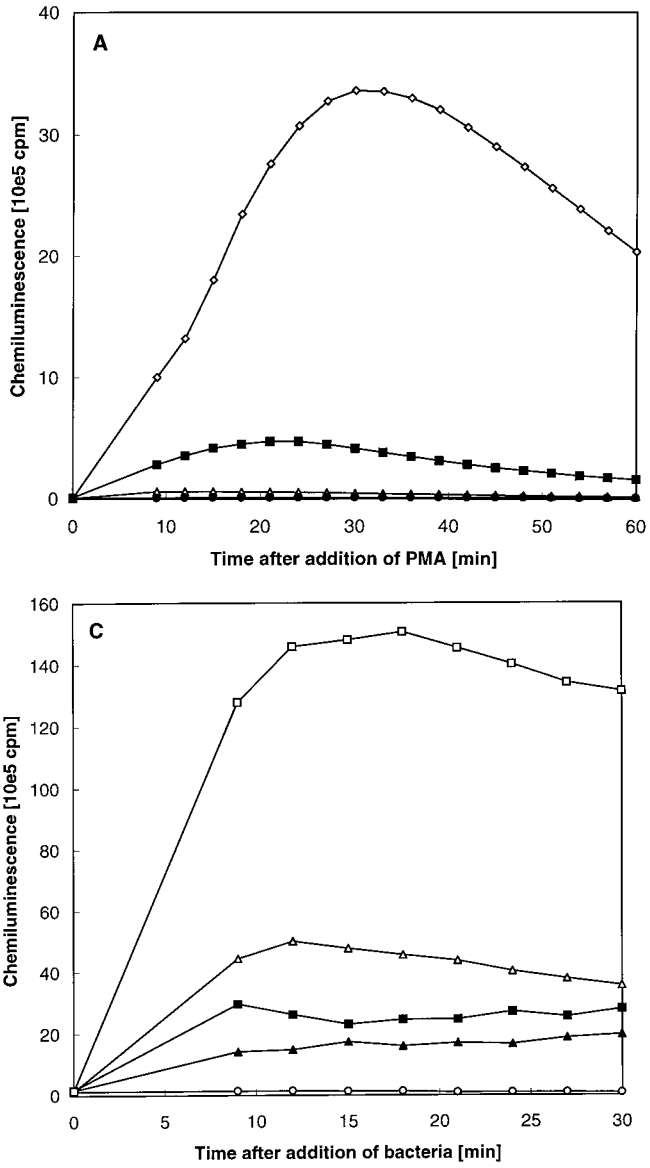

FIG. 4. Luminol-enhanced chemiluminescence of in vitro-differentiated JOSK-M cells and primary monocytes. (A) JOSK-M cells were differentiated with 100 nM retinoic acid and 10 nM bufalin for 5 (◇), 3 (■), or 2 (△) days. Cells (2×10^6/ml) were incubated in CL-PBS (see Materials and Methods). After addition of PMA (0.1 μg/ml), luminol-enhanced chemiluminescence was recorded for 60 min. Control cells received 0.1% ethanol (●). (B) JOSK-M cells were differentiated with 100 nM retinoic acid and 10 nM bufalin for 5 days and suspended in CL-PBS (2×10^6/ml). After stimulation of the cells with Opa_{52}-expressing (△) or Opa^- (▲) *N. gonorrhoeae*, with *N. sicca* (■), or with *N. cinerea* (□), luminol-enhanced chemiluminescence was recorded for 60 min. Control cells (○) were left without bacteria. (C) Primary human peripheral blood mononuclear cells were suspended in CL-PBS (2×10^6/ml) and incubated with Opa_{52}-expressing (△) or Opa^- (▲) *N. gonorrhoeae*, with *N. sicca* (■), or with *N. cinerea* (□), and luminol-enhanced chemiluminescence was recorded for 30 min. Control cells (○) were left without bacteria. The graphs show representative results of one of three experiments.

lation led to a marked increase in luminol-enhanced chemiluminescence (Fig. 4A). These results were confirmed by staining cell preparations for superoxide anions, the starting material of the oxidative burst generated by NADPH oxidase. Only 7% of undifferentiated JOSK-M cells were able to reduce NBT after PMA stimulation, whereas 4 days after incubation with retinoic acid and bufalin, 64% of the cells generated reactive oxygen derivatives in this assay (data not shown).

Differentiated JOSK-M cells were not only able to mount an oxidative response after stimulation with the soluble protein kinase C-stimulating agent PMA, but they also responded to particulate stimuli. If JOSK-M cells were incubated with gonococci, the generation of an oxidative burst depended on Opa protein expression of the bacteria. Opa_{52}-expressing bacteria induced a continuous increase in luminol-dependent chemiluminescence over a 1-h incubation period (Fig. 4B). The peak luminescence of *N. gonorrhoeae*-stimulated cells reached after 50 to 60 min did not reach the levels of PMA-stimulated JOSK-M cells. In contrast, if the cells were challenged with the same amount of Opa^- gonococci, no response could be detected and chemiluminescence remained at the background levels of nonstimulated control cells. Interestingly, when we used *N. sicca* and *N. cinerea*, two nonpathogenic commensal members of the genus *Neisseria*, JOSK-M cells reacted with a strong increase in reactive oxygen generation (Fig. 4B).

A similar reaction profile was observed after stimulation of human peripheral blood monocytes. Although these cells reached their maximal activity within 10 to 20 min after addition of the bacteria, the intensity of the chemiluminescence signal obtained after incubation with commensal *N. cinerea* or Opa^+ *N. gonorrhoeae* was almost identical to that obtained with JOSK-M cells (Fig. 4C). Again, *N. cinerea* elicited a much stronger oxidative response than did opaque gonococci, whereas nonopaque, nonpiliated *N. gonorrhoeae* only marginally stimulated primary cells. The luminol-dependent chemiluminescence of primary monocytes differed from that of in vitro-differentiated JOSK-M cells with respect to *N. sicca*, which caused primary monocytes to respond with only a slight increase in oxidative metabolism.

Influence of gonococcal infection on MHC class II surface expression. Monocytes may play an important role in the response to gonococcal infection not only by phagocytosis and killing of bacteria (7, 26) but also by initiating a specific immune response due to their antigen-presenting capacity. Since JOSK-M cells express major histocompatibility complex (MHC) class II molecules, we used this new model to investigate if *N. gonorrhoeae* has an influence on the antigen presentation capacity of infected cells. As illustrated in Table 2, the

TABLE 2. Influence of gonococci on MHC II expression on the surface of JOSK-M cells[a]

Cell status	Uninfected cells		Infected cells	
	% HLA-DR positive	MFI	% HLA-DR positive	MFI
Undifferentiated	99	623	ND	ND
Retinoic acid-bufalin differentiated	95	492	93	518

[a] Cells were treated for 4 days with retinoic acid and bufalin or mock treated with ethanol. Differentiated cells were infected with Opa^+ gonococci, and cells were stained with anti-HLA-DR antibody and dichlorotriacyl-aminofluorescein-conjugated goat-anti-mouse $F(ab')_2$ fragments as described in Materials and Methods. Cells were analyzed with a Becton-Dickinson FACSort by gating 10^4 cells according to their forward and sideward scatter, and the percentage of HLA-DR-positive cells and their MFI were determined. ND, not determined.

differentiation of JOSK-M cells with retinoic acid-bufalin slightly reduced the proportion of HLA-DR-positive cells (99% of untreated cells were positive, compared to 95% after differentiation), and it significantly decreased the amount of surface-exposed molecules as indicated by the lower mean fluorescence intensity (MFI) of differentiated cells (MFI of 492 compared to an MFI of 623 in undifferentiated cells). Infection of differentiated cells by gonococci had no influence on MHC surface expression. Neither the mean fluorescence (MFIs of 518 and 492, respectively) nor the proportion of HLA-DR-positive cells was altered by infection of JOSK-M cells with gonococci (Table 2).

DISCUSSION

Since pathogenic neisseriae are extremely host specific, investigations depend almost completely on the use of in vitro models involving human material. With respect to initial infection processes like meningococcal and gonococcal adhesion or invasion of epithelial and endothelial cells, permanent cell lines have already proven to be indispensable tools (2, 12, 16, 20, 40, 43). On the other hand, studies concerning the interaction of neisseriae with phagocytic cells have so far been limited to primary human blood cells, a highly variable source (15, 22, 26, 28). This situation makes it difficult to compare results obtained sequentially with blood from different donors. Also, interesting questions regarding gonococci and antigen presentation or investigations requiring transfection of cells have not been addressed so far because of the lack of a permanent human phagocytic cell line that interacts with these microorganisms. Our initial attempts to solve this problem by the use of human leukemia-derived myelomonocytic, monocytic, and histiocytic cell lines failed. As is described for undifferentiated myeloid HL-60 cells (9), only a very low degree of interaction between gonococci and these permanent cell lines can be detected (Fig. 1).

Because leukemic cell lines are often arrested at precursor stages of mature granulocytes and monocyte-macrophage development, it is perhaps not surprising that they are unable to carry out phagocytosis, a characteristic feature of terminally differentiated cells (39). Several chemical agents, cytokines, vitamin analogs, and antileukemia drugs have been shown to induce the differentiation of human leukemia-derived cell lines (5, 27, 30, 37, 45). To obtain phagocytic antigen-presenting cells, we therefore incubated the MHC class II-positive cell lines JOSK-M and MonoMac-6 with a selection of these factors.

Although TNF-α, IFN-γ, GM-CSF, bufalin, retinoic acid, and vitamin D_3 have all been reported to promote monocyte-macrophage differentiation (5, 27, 30, 37, 45, 46), only treatment with the latter two compounds resulted in clearly enhanced phagocytic activity towards gonococci. Interestingly, bufalin also had a strong potentiating effect on JOSK-M differentiation when used together with retinoic acid or vitamin D_3 but lacked any effect when used alone. The incubation of JOSK-M cells with retinoic acid and bufalin led to highly phagocytic cells that internalized gonococci to the same extent as primary monocytes from human blood (Fig. 1). The synergistic effect of bufalin in combination with other monocyte-macrophage differentiation promoters has been recently described. This strategy has been used to transform human histiocytic U937 leukemia cells into phagocytic monocyte-macrophage-like cells (6, 46), although higher doses have been shown to induce apoptosis in human HL60 and ML1 cells (21).

Farrell and Rest have analyzed the interactions which occur between *N. gonorrhoeae* and HL60 cells (9). Dimethyl sulfoxide-treated, as well as PMA-treated, HL60 cells, which are known to resemble either mature granulocytes or mature monocytes in many respects, were reported not to interact with gonococci, leading the investigators to conclude that both naive and in vitro-differentiated HL60 cells lack receptors responsible for the uptake of unopsonized Opa^+ gonococci. Our present results, however, indicate both that various cell lines interact to different degrees with *N. gonorrhoeae* and that this interaction is dependent on the applied differentiation-inducing agent.

For several gonococcal isolates, it has been shown that while some Opa proteins confer the ability to interact with phagocytes, others fail to promote adherence to or uptake by human monocytes or neutrophils (16, 28, 42). Especially the expression of one previously characterized Opa protein, Opa_{52}, led to enhanced phagocytosis of bacteria by JOSK-M cells. A variant of this Opa protein from the same strain has previously been shown to induce an oxidative burst when expressed in *Escherichia coli* (1) and to lead to enhanced association with polymorphonuclear granulocytes from human blood (16). Interestingly, piliated Opa^- gonococci are phagocytosed more efficiently than some Opa variants, but the expression of Opa_{52} still increased the uptake of piliated bacteria by the human cells.

If a distinct receptor for the uptake of these bacteria exists, then JOSK-M cells should be a useful tool for identifying this molecule, since they phagocytose *N. gonorrhoeae* by a process that depends on the specific Opa protein variant that is expressed. Interestingly, although MonoMac-6 and JOSK-M cells show similar overall patterns of stimulation with the inducers, these cell lines exhibit different degrees of interaction with gonococci. In line with Rest's results, it could be speculated that both cell lines possess a putative receptor(s) for Opa^+ *N. gonorrhoeae* but express them at different levels.

The increased phagocytic activity of JOSK-M cells following bufalin-retinoic acid treatment is paralleled by another gain of function. During differentiation by these two agents, the oxidative burst capacity of JOSK-M cells increased with time. Whereas undifferentiated cells responded to neither PMA nor other known stimuli like *N*-formylmethionyl-leucyl-phenylalanine zymosan, or bacteria (data not shown), incubation with retinoic acid and bufalin led to cells that were able to yield a detectable luminol-enhanced chemiluminescence response. Additionally, the in vitro-differentiated permanent cells showed the same pattern of oxidative burst activity in response to *N. gonorrhoeae* as primary human monocytes (10, 28). In both cases, opaque gonococci stimulated a significant oxidative burst, whereas nonopaque, nonpiliated gonococci only slightly

activated the phagocytes. In contrast to JOSK-M cells, primary monocytes responded immediately after the addition of bacteria, but the intensities of oxidative metabolism were strikingly similar in the two cell types.

In this context, it is important to note that both the oxidative burst capacity and the phagocytic activity of JOSK-M cells matured in the same time course. Since both activities are regulated by small GTP-binding proteins, these changes may reflect an alteration of signal transduction upon the maturation of these cells (4).

Differentiated JOSK-M cells also responded to nonpathogenic neisseriae with the generation of a strong oxidative burst. Since *N. cinerea* induced an even stronger reaction than opaque *N. gonorrhoeae* in both JOSK-M cells and primary monocytes, we speculated that this commensal is also phagocytosed more efficiently than gonococci in our assay. However, we detected *N. cinerea* in only a minority of JOSK-M cells (14a). Since both phagocytosis and the oxidative burst depend on recognition and close interaction of eukaryotic cells and bacteria, Opa^+ gonococci seem to possess a mechanism that allows tight adherence followed by phagocytosis without adequate stimulation of the oxidative burst machinery. It has been shown that the gonococcal outer membrane porin PorB (P.I.) can reduce the oxidative burst by interfering with the release of myeloperoxidase from granulocyte azurophil granules (13, 18a). In contrast to PorB from commensal species, this porin has also been shown to translocate from bacteria into artificial and host cell membranes (19, 44) and to bind ATP (34). The striking differences between gonococci and commensal species with regard to the relationship between luminol-enhanced chemiluminescence and phagocytosis could be due to the action of PorB inserted into the host cell membrane after Opa-mediated intimate contact. It should be very interesting to study this process in more detail by using in vitro-differentiated JOSK-M cells.

Several studies have demonstrated that pathogens can affect the antigen-presenting and accessory functions of monocytes due to changes in the expression of molecules important for their interaction with T cells, presumably by distortion of signaling pathways that activate monocytes (reviewed in reference 31). In *Mycobacterium tuberculosis*-infected monocytes, it has been shown by Gercken and coworkers (11) that HLA-DR expression on the cell surface was markedly reduced following uptake of the mycobacteria. This reduction was accompanied by an impaired ability of the monocytes to stimulate T cells. Since JOSK-M cells are capable of antigen presentation, we wondered whether phagocytosis of *N. gonorrhoeae* alters the surface expression of MHC class II molecules. We could not detect altered surface expression of HLA-DR after phagocytosis of *N. gonorrhoeae*, whereas the in vitro differentiation of JOSK-M cells slightly reduced the proportion of HLA-DR-positive cells and the amount of surface-exposed molecules (Table 2).

Taken together, our results demonstrate that in vitro-differentiated human myelomonocytic JOSK-M cells provide a suitable model for the study of a variety of aspects within the framework of the gonococcus-phagocyte interaction. They not only resemble primary monocytes regarding cell type-specific functions, but they also show the same pattern of interaction with *N. gonorrhoeae* as primary human cells. This system offers several advantages for detailed studies on the cellular level, since the continuous supply of standardized and phenotypically similar human cells whose activation and differentiation status can be manipulated and experimentally controlled will enable investigations requiring large and/or homogeneous batches of cells or those involving stable and transient transfection.

ACKNOWLEDGMENTS

We thank L. Huber for his interest and helpful discussions. We are grateful to T. Friede and H. G. Rammensee for the provision of antibodies, to Petra Haerder for technical assistance, and to S. Gray-Owen for suggestions on the manuscript.

This work was supported in part by BMFT grant 01-KI-8920/5 and by the Fonds der Chemischen Industrie.

REFERENCES

1. **Belland, R. J., T. Chen, J. Swanson, and S. H. Fischer.** 1992. Human neutrophil response to recombinant neisserial Opa proteins. Mol. Microbiol. **6:**1729–1737.
2. **Bessen, D., and E. C. Gotschlich.** 1986. Interactions of gonococci with HeLa cells: attachment, detachment, replication, penetration, and the role of protein II. Infect. Immun. **54:**154–160.
3. **Blake, M. S., and L. M. Wetzler.** 1995. Vaccines for gonorrhea: where are we on the curve? Trends Microbiol. **3:**469–474.
4. **Bokoch, G. M., and U. G. Knaus.** 1994. The role of small GTP-binding proteins in leukocyte function. Curr. Opin. Immunol. **6:**98–105.
5. **Breitman, T. R., S. E. Selonick, and S. J. Collins.** 1980. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. Proc. Natl. Acad. Sci. USA **77:**2936–2940.
6. **Desjardins, M., J. E. Celis, G. van Meer, H. Dieplinger, A. Jahraus, and L. A. Huber.** 1994. Molecular characterization of phagosomes. J. Biol. Chem. **269:**32194–32200.
7. **Drutz, D. J.** 1978. Intracellular fate of *Neisseria gonorrhoae*, p. 232–235. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
8. **Elkins, C., and R. F. Rest.** 1990. Monoclonal antibodies to outer membrane protein PII block interactions of *Neisseria gonorrhoeae* with human neutrophils. Infect. Immun. **58:**1078–1084.
9. **Farrell, C. F., and R. F. Rest.** 1990. Up-regulation of human neutrophil receptors for *Neisseria gonorrhoeae* expressing PII outer membrane proteins. Infect. Immun. **58:**2777–2784.
10. **Fischer, S. H., and R. F. Rest.** 1988. Gonococci possessing only certain PII outer membrane proteins interact with human neutrophils. Infect. Immun. **56:**1574–1579.
11. **Gercken, J., J. Pryjma, M. Ernst, and H.-D. Flad.** 1994. Defective antigen presentation by *Mycobacterium tuberculosis*-infected monocytes. Infect. Immun. **62:**3472–3478.
12. **Grassme, H. U. C., R. M. Ireland, and J. P. M. van Putten.** 1996. Gonococcal opacity protein promotes bacterial entry-associated rearrangements of the epithelial cell actin cytoskeleton. Infect. Immun. **64:**1621–1630.
13. **Haines, K. A., L. Yeh, M. S. Blake, P. Cristello, H. Korchak, and G. Weissmann.** 1988. Protein I, a translocatable ion channel from *Neisseria gonorrhoeae*, selectively inhibits exocytosis from human neutrophils without inhibiting O_2^- generation. J. Biol. Chem. **263:**945–951.
14. **Handsfield, H. H.** 1990. *Neisseria gonorrhoeae*, p. 1613–1631. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases. Churchill Livingstone, New York, N.Y.

14a. **Hauck, C. R., and T. F. Meyer.** Unpublished data.

15. **Krieger, A. G., N. L. Schiller, and R. B. Roberts.** 1980. Gonococci-human polymorphonuclear leukocyte interactions: metabolic studies associated with attachment and ingestion. Infect. Immun. **28:**991–1000.
16. **Kupsch, E.-M., B. Knepper, T. Kuroki, I. Heuer, and T. F. Meyer.** 1994. Variable opacity (Opa) outer membrane proteins account for the cell tropisms displayed by *Neisseria gonorrhoeae* for human leukocytes and epithelial cells. EMBO J. **12:**641–650.
17. **Kupsch, E. M., D. Aubel, C. P. Gibbs, A. F. Kahrs, T. Rudel, and T. F. Meyer.** 1996. Construction of the Hermes shuttle vectors—a versatile system useful for genetic complementation of transformable and non-transformable *Neisseria* mutants. Mol. Gen. Genet. **250:**558–569.
18. **Lampson, L. A., and R. Levy.** 1980. Two populations of Ia-like molecules on a human B cell line. J. Immunol. **125:**293–299.

18a. **Lorenzen, D., et al.** Unpublished data.

19. **Lynch, E. C., M. S. Blake, E. C. Gotschlich, and A. Mauro.** 1984. Studies of porins: spontaneously transferred from whole cells and reconstituted from purified proteins of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Biophys. J. **45:**104–107.
20. **Makino, S., J. P. M. van Putten, and T. F. Meyer.** 1991. Phase variation of the opacity outer membrane protein controls invasion by *Neisseria gonorrhoeae* into human epithelial cells. EMBO J. **10:**1307–1315.
21. **Masuda, Y., N. Kawazoe, S. Nakajo, T. Yoshida, Y. and K. Kuroiwa.** 1995. Bufalin induces apoptosis and influences the expression of apoptosis-related genes in human leukemia cells. Leukemia Res. **19:**549–556.
22. **McNeil, G., M. Virji, and E. R. Moxon.** 1994. Interactions of *Neisseria meningitidis* with human monocytes. Microb. Pathog. **16:**153–163.
23. **Meyer, T. F., E. Billyard, R. Haas, S. Storzbach, and M. So.** 1984. Pilus genes of *Neisseria gonorrhoeae*: chromosomal organization and DNA sequence. Proc. Natl. Acad. Sci. USA **81:**6110–6114.

24. **Meyer, T. F., C. P. Gibbs, and R. Haas.** 1990. Variation and control of protein expression in *Neisseria*. Annu. Rev. Microbiol. **44:**451–477.
25. **Meyer, T. F., J. Pohlner, and J. P. M. van Putten.** 1994. Biology of the pathogenic *Neisseria*. Curr. Top. Microbiol. Immunol. **192:**283–317.
26. **Mezzatesta, J. R., and R. F. Rest.** 1983. Phagocytic killing of *Neisseria gonorrhoeae* by human monocytes. Infect. Immun. **42:**99–105.
27. **Miyaura, C., E. Abe, T. Kuribayashi, H. Tanaka, K. Konno, Y. Nishii, and T. Suda.** 1981. 1-Alpha, 25-dihydroxyvitamin D_3 induces differentiation of human myeloid leukemia cells. Biochem. Biophys. Res. Commun. **102:**937–943.
28. **Naids, F. L., and R. F. Rest.** 1991. Stimulation of human neutrophil oxidative metabolism by nonopsonized *Neisseria gonorrhoeae*. Infect. Immun. **59:**4383–4390.
29. **Ohta, M., Y. Furukawa, C. Ide, N. Akiyama, T. Utakoji, Y. Miura, and M. Saito.** 1986. Establishment and characterization of four human monocytoid leukemia cell lines (JOSK-I, -S, -M and -K) with capabilities of monocyte-macrophage lineage differentiation and constitutive production of interleukin 1. Cancer Res. **46:**3067–3074.
30. **Perussia, B., E. T. Dayton, V. Fanning, P. Thiagarajan, J. Hoxie, and G. Trinchieri.** 1983. Immune interferon and leukocyte-conditioned medium induce normal and leukemic myeloid cells to differentiate along the monocytic pathway. J. Exp. Med. **158:**2058–2080.
31. **Reiner, N. E.** 1994. Altered cell signaling and mononuclear phagocyte deactivation during intracellular infection. Immunol. Today **15:**374–381.
32. **Rest, R. F., and W. M. Shafer.** 1989. Interactions of *Neisseria gonorrhoeae* with human neutrophils. Clin. Microbiol. Rev. **2**(Suppl.)**:**S83–S91.
33. **Robertson, B. D., and T. F. Meyer.** 1992. Genetic variation in pathogenic bacteria. Trends Genet. **8:**422–427.
34. **Rudel, T., A. Schmid, R. Benz, H.-A. Kolb, F. Lang, and T. F. Meyer.** 1996. Modulation of Neisseria porin (PorB) by cytosolic ATP/GTP of target cells: parallels between pathogen accommodation and mitochondrial endosymbiosis. Cell **85:**1–20.
35. **Shafer, W. M., and R. F. Rest.** 1989. Interactions of gonococci with phagocytic cells. Annu. Rev. Microbiol. **43:**121–145.
36. **Sundström, C., and K. Nilsson.** 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int. J. Cancer **17:**565–577.
37. **Trinchieri, G., M. Kobayashi, M. Rosen, R. Loudon, M. Murphy, and B. Perussia.** 1986. Tumor necrosis factor and lymphotoxin induce differentiation of human myeloid cell lines in synergy with immune interferon. J. Exp. Med. **164:**1206–1225.
38. **Tsuchiya, S., M. Yamabe, Y. Yamaguchi, Y. Kobayashi, T. Konno, and K. Tada.** 1980. Establishment and characterization of a human acute monocyte leukemia cell line (THP-1). Int. J. Cancer **26:**171–176.
39. **Turpin, J. A., and G. Lopez-Berestein.** 1993. Differentiation, maturation, and activation of monocytes and macrophages: functional activity is controlled by a continuum of maturation, p. 71–99. *In* G. Lopez-Berestein and J. Klostergaard (ed.), Mononuclear phagocytes in cell biology. CRC Press, Inc., Boca Raton, Fla.
40. **van Putten, J. P. M.** 1993. Phase variation of lipopolysaccharide directs interconversion of invasive and immuno-resistant phenotypes of *Neisseria gonorrhoeae*. EMBO J. **12:**4043–4051.
41. **Veale, D. R., H. Finch, and H. Smith.** 1976. Penetration of penicillin into human phagocytes containing *Neisseria gonorrhoeae*: intracellular survival and growth at optimum concentrations of antibiotic. J. Gen. Microbiol. **95:**353–363.
42. **Virji, M., and J. E. Heckels.** 1986. The effect of protein II and pili on the interaction of *Neisseria gonorrhoeae* with human polymorphonuclear leukocytes. J. Gen. Microbiol. **132:**503–512.
43. **Weel, J. F. L., and J. P. M. van Putten.** 1988. Ultrastructural localization of gonococcal antigens in infected epithelial cells as visualized by post-embedding immunoelectron microscopy. Microb. Pathog. **4:**213–222.
44. **Weel, J. F. L., and J. P. M. van Putten.** 1991. Fate of the major outer membrane protein P.IA in early and late events of gonococcal infection of epithelial cells. Res. Microbiol. **142:**985–993.
45. **Zhang, L. S., K. Nakaya, T. Yoshida, and Y. Kuroiwa.** 1991. Bufalin as a potent inducer of differentiation of human myeloid leukemia cells. Biochem. Biophys. Res. Commun. **178:**686–693.
46. **Zhang, L. S., K. Nakaya, T. Yoshida, and Y. Kuroiwa.** 1992. Induction by bufalin of differentiation of human leukemia cells HL60, U937, and ML1 toward macrophage/monocyte-like cells and its potent synergistic effect on the differentiation of human leukemia cells in combination with other inducers. Cancer Res. **52:**4634–4641.
47. **Ziegler-Heitbrock, H. W. L., E. Thiel, A. Fütterer, V. Herzog, A. Wirtz, and G. Riethmüller.** 1988. Establishment of a human cell line (MonoMac-6) with characteristics of mature monocytes. Int. J. Cancer **41:**456–461.

Editor: P. J. Sansonetti