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Monocyte–T-cell interactions in pokeweed mitogen-activated cultures

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SUMMARY

Monocyte–T-cell interactions were studied in pokeweed mitogen (PWM)-activated cell cultures. We addressed the question of monocyte changes in PWM-stimulated cultures of T cells and monocytes and found, by flow cytometric analysis, that PWM activation led to a loss of cells with monocyte or macrophage phenotype (CD14, HLA-DR, HLA-DQ) within 48 hr of culture in the presence of T cells ($CD4^+$ T cells), but not in cultures of pure monocytes. Chemiluminescence measurements revealed that phagocytic stimulation of monocytic superoxide release was impaired in PWM-stimulated cultures of monocytes plus T cells, but not in PWM-stimulated cultures of pure monocytes. Furthermore, PWM induced the secretion of interferon-gamma (IFN-γ) in primary cultures of T cells supplemented with 20% of monocytes, whereas in subsequent secondary cultures of these cells PWM induced IFN-γ only when monocytes were added. We conclude from these flow cytometric and functional analyses that monocytes are efficiently eliminated from PWM-activated T-cell/monocyte cultures by $CD4^+$ T lymphocytes.

INTRODUCTION

Monocyte–T-cell interactions play an important immunoregulatory role in pokeweed mitogen (PWM)-activated cultures of human lymphocytes (ElMasry *et al.*, 1986; Pryjma *et al.*, 1986, 1989; Zembala *et al.*, 1984). In particular, it has been shown that such interactions are important during activation of T cells with suppressive function (ElMasry *et al.*, 1986). In PWM-stimulated cultures, activation and proliferation of T cells is required before suppressor activity can be demonstrated (Pryjma *et al.*, 1986; Waldmann & Broder, 1982). This process is monocyte-dependent, but the precise role and the stage at which monocytes are needed are unknown (Thiele *et al.*, 1983). Furthermore, the possible influence of activated T cells on monocytes in such cultures is not clear, although preliminary observations suggest that the immunoregulatory (suppressive) function of monocytes depends on the presence of T cells (Pryjma *et al.*, 1986). It seems reasonable to expect that in response to direct or indirect signals from T cells changes of monocyte phenotype would occur. Such changes are very likely, since at least one T-cell product, interferon-gamma (IFN-γ), is a potent monocyte activator and modulates expression of several antigens on the monocyte surface (Gonwa *et al.*, 1986; Hoover & Meltzer, 1989; Jayaram *et al.*, 1989). Interestingly, in PWM-stimulated cultures the production of IFN-γ is strongly enhanced by monocytes (J. Pryjma *et al.*, manuscript in preparation). Among phenotypic changes induced in monocytes by IFN-γ, the expression of HLA class II determinants seems to be particularly interesting. These determinants, apart from being necessary for antigen presentation, were shown to be important in mitogen-driven T-cell proliferation and are activators of immunoregulatory T cells (Gansbacher & Zirr, 1988; Nieda *et al.*, 1988).

Therefore, the aim of the present study was to follow the expression of HLA class II determinants on monocytes during culture with T cells in the presence of PWM. During these studies we noticed that the number of monocytes in activated cultures was dramatically reduced during the first 48 hr of culture and that this reduction was probably caused by activated CD4 lymphocytes.

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MATERIALS AND METHODS

Cell populations

Peripheral blood mononuclear cells (PBMC) were isolated by standard lymphoprep (Nyegaard, Norway) gradient from the EDTA or heparin-treated blood of healthy donors. PBMC were separated into T and non-T cells by rosetting with neuraminidase-treated sheep erythrocytes and subsequent Lymphoprep gradient centrifugation. Rosetting cells (T lymphocytes) were recovered from the pellet by osmotic shock, washed twice with Hanks' buffer, and finally adjusted to the concentration of 1×10^6/ml in the complete medium RPMI-1640 supplemented with 10% foetal calf serum (FCS), 2 mM L-glutamine, and antibiotics (all reagents from Gibco, Karlsruhe, FRG). Non-T

cells were adjusted to the concentration of 2×10^6/ml in RPMI medium and incubated for 1 hr on FCS-precoated plastic Petri dishes. Thereafter, non-adherent cells were removed, plates washed several times with warm medium, and adherent cells (monocytes) detached from the plastic into ice-cold medium with a rubber policeman. These cells were 83–92% pure monocytes, as judged from staining with non-specific esterase. In some experiments PBMC were suspended in Hanks' buffer and underwent counter-current centrifugal elutriation (Beckman JE-6B Elutriation System, Beckman Instruments, München, FRG). Twenty cell fractions were collected. Monocyte enrichment in the last fraction was confirmed by non-specific esterase staining (85–95% positive) and/or expression of CD14 antigen and HLA-DR (80–90% Leu-M3 and 70–95% HLA-DR positive). Cells collected in fractions 4–6 (which contained 80–90% Leu-4-positive cells) were pooled and rosetted with neuraminidase-pretreated sheep erythrocytes to isolate T cells. Such a preparation contained cells which were more than 95% $CD3^+$ ($Leu\text{-}4^+$) and contained as a rule less than 1% of esterase-positive cells.

Isolation of T-cell subsets

In some experiments T cells were depleted of $CD8^+$ subsets by panning on plastic plates with bound OKT8 (Ortho, Heidelberg, FRG) monoclonal antibody. The resulting non-adherent population, when re-analysed by indirect immunofluorescence, was enriched up to 90–94% and was contaminated with less than 3% of $CD8^+$ cells, as judged from labelling with FITC-conjugated Leu-2a monoclonal antibody (Becton-Dickinson, Heidelberg, FRG).

Mitogen and other reagents used

PWM (Seromed, Biochem, Berlin, FRG) was used in culture at a final concentration of 1 μg/ml. In some experiments PWM (Gibco) at a final dilution of 1:200 was used. The results were the same regardless of mitogen source, and this information will not be included in the Results section. These concentrations of lectin induce, in our hands, an optimal response measured by immunoglobulin secretion in cultures of mononuclear cells. Recombinant interleukin-2 (IL-2) and interferon-gamma (IFN-γ) were kindly supplied by Glaxo (Geneva, Switzerland) and were used accordingly at concentrations of 50 and 100 U/ml.

Cell cultures

Isolated monocytes or co-cultures of T cells and monocytes mixed in a 4:1 ratio were cultured in 1-ml aliquots in flat-bottomed 24-well culture plates (Nunclon, Nunc, Denmark) in the presence of PWM. Unless otherwise stated, after 2 days the plates were transferred on ice for 30 min, and thereafter the cultured cells were collected. As a rule, adherent cells were detached from the surface with a rubber policeman. Finally, cells were suspended in ice-cold phosphate-buffered saline (PBS) and labelled with monoclonal antibodies (see below).

Culture supernatants

In some cases, at the indicated times, the culture supernatant was collected and, when required, replaced with fresh complete medium. Supernatants were passed through Millipore filter and stored at −20° until use.

Phenotypic analysis

After washing once with PBS containing azide, cells were labelled with a panel of commercially available monoclonal antibodies (mAb). The phycoerythrin (PE)- or fluorescein (FITC)-conjugated Leu-2a, Leu-4, Leu-M3, anti-HLA-DR, anti-HLA-DQ, mouse IgG1 isotype control, and unlabelled anti-HLA-DP were all from Becton-Dickinson. As a second antibody FITC-labelled $F(ab')_2$ fragments of goat anti-mouse IgG (H + L) (Dianova, Hamburg, FRG) was used. The labelled cells were analysed by flow cytometry using FACScan or FACStar Plus (Becton-Dickinson). Data were acquired ungated and, thereafter, unless otherwise stated, analysed in a gate which, according to forward and side scatter signals, corresponded to monocytes. These will be referred to as 'gated monocytes', since out of a mixture of monocytes and T cells over 90% of gated cells were CD14 (Leu-M3) positive at the time of culture setting. Since occasionally cultured cells expressed some autofluorescence, the conditions of analysis (position of the cursor) were set in a way that the percentage of positive cells in control samples was 1–5%, and this value was as a rule subtracted.

Chemiluminescence (CL) measurements

These were performed as already described (Ernst *et al.*, 1984). Aliquots of 4×10^4 monocytes in 0·2 ml of complete medium were cultured alone or with $1{\cdot}6 \times 10^5$ T cells directly in round-bottomed polystyrene measuring vials for the indicated time-period. Thereafter, 0·5 hr before CL measurement, 0·1 ml of culture medium was replaced with 0·3 ml of warm chemiluminescence medium (CL medium; Boehringer, Mannheim, FRG). The CL response to latex beads (Sigma, Deisenhofen, FRG) was measured using luminol as luminescence indicator and a 6-channel measuring device (Biolumat LB 9505, Berthold, Wildbad, FRG), which allowed the simultaneous CL measurement of six samples at 37°. In time–course experiments, the results were expressed as comparison (percentage of the response) of experimental and control samples, both expressed as integral c.p.m. per 10 min.

IFN-γ measurement

The concentration of IFN-γ was measured using the ELISA test kit kindly provided by Dr Gallati (Hoffmann-La Roche, Basle, Switzerland). In brief, monoclonal anti-IFN-γ antibody (Clone 69, 5 μg/ml in 0·1 M bicarbonate buffer, pH 9·5), from Dr Gallati was added to the wells of polystyrene microtitre plates (F-shape; Greiner, Nürtingen, FRG) and allowed to bind overnight at room temperature. Plates were then rinsed five times with distilled water, and the wells were filled for at least 48 hr at 4° with 0·2 M Tris/HCl buffer, pH 7·5, containing 1% bovine serum albumin (BSA). After washing, 50-μl aliquots of samples to be tested and rIFN-γ as a standard were added in serial twofold dilutions in 0·1 M PBS containing 0·5% BSA. Peroxidase-conjugated anti-IFN-γ monoclonal antibody, provided by Dr Gallati (Clone 123, 50 μl/well), was added at the same time. After 24 hr of incubation at 4° and five further washes with distilled water, plates were developed with 100 μl/well of 3,5,3′, 5′-tetramethylbenzidine in 0·2 M potassium citrate buffer, pH 3·95, supplemented with 0·003% H_2O_2. After 20–25 min the reaction was stopped by adding to each well 50 μl of 1 N H_2SO_4 and monitored at 450 nm with a spectrophotometer. The results were expressed in U/ml calculated from the dilution curve of the rIFN-γ standard.

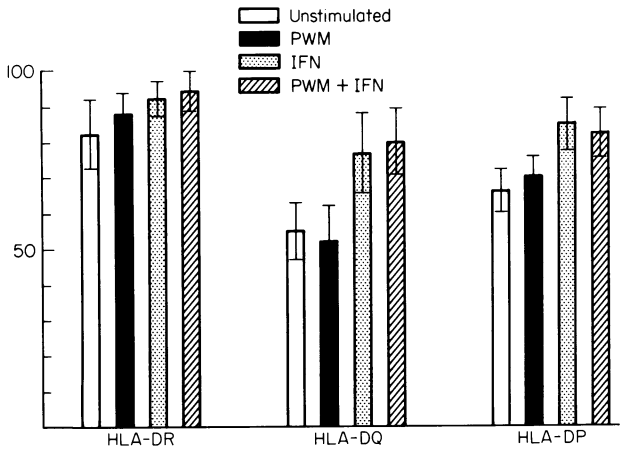

Figure 1. Expression of HLA class II antigens in cultures of monocytes. Monocytes, 1×10^6/ml in 1-ml aliquots, were cultured for 48 hr as indicated. Thereafter, cells were labelled with mAb against HLA-DR, HLA-DQ and HLA-DP and analysed in a flow cytometer using a pre-set analysis gate for monocytes. As a rule, after cell debris was excluded, more than 80% of cells fitted the gate. Data are mean and SD from six experiments. The results were the same irrespective of the method of monocyte purification, adherence (two experiments) or centrifugal elutriation (four experiments).

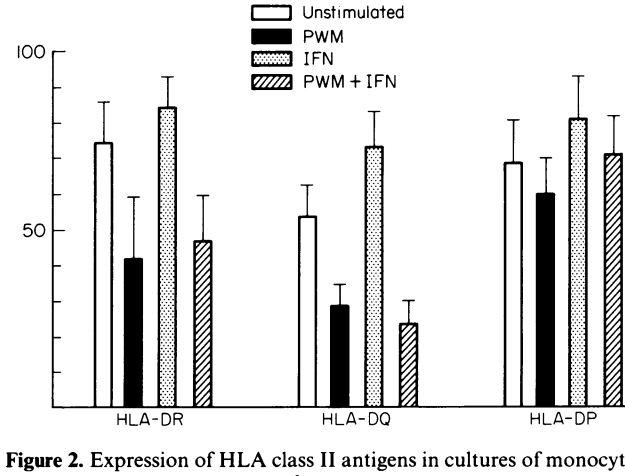

Figure 2. Expression of HLA class II antigens in cultures of monocytes and T cells. Monocytes, 2×10^5, were cultured together with 8×10^5 T cells in the presence of indicated stimuli. The results (mean and SD of 14 experiments) are from an analysis gate which included 14–30% of all cells. Data of experiments where monocytes were separated by adherence and elutriation were pooled.

RESULTS

Expression of HLA class II determinants on monocytes activated with PWM

Isolated monocytes were cultured either alone or with T cells in the presence or absence of PWM. To some cultures IFN-γ (100 U/ml) was also added. After 48 hr cells were harvested, labelled with anti-class II mAb, and analysed by flow cytometry. The gate for monocyte analysis was set which included cells with high forward and 90% scatter signal—'gated monocytes' (see the Materials and Methods). Addition of PWM to monocytes cultured alone did not result in a significant change of the proportion of cells with class II determinants (Fig. 1) and caused no apparent change in their expression, as judged from recorded intensity of fluorescence (not shown). IFN-γ added to cultures of unstimulated and PWM-activated monocytes significantly increased the proportion of cells which expressed HLA-DQ, HLA-DP and, usually (in four out of six experiments), HLA-DR class II determinants (Fig. 1). In addition, the intensity of fluorescence was higher on monocytes precultured with IFN-γ, indicating that the amount of detected antigen was increased on their surface (not shown). The increase in the proportion of cells which express class II determinants after incubation with IFN-γ is in agreement with reports of others (Gonwa *et al.*, 1986), and our data show in addition that the presence of PWM does not interfere with the IFN-γ-mediated effect.

Expression of HLA class II determinants on gated monocytes after activation with PWM in the presence of T lymphocytes

As shown in Fig. 2, in PWM-activated cultures of monocytes and T cells the proportion of HLA-DR- and DQ-positive cells among gated monocytes was markedly reduced. Addition of IFN-γ to these cultures had no effect on the observed reduction of HLA-DQ expression, although this lymphokine added to unstimulated cultures of monocytes and T cells reproducibly enhanced the expression of HLA-DR, HLA-DQ and HLA-DP. To find out whether T-cell products other than IFN-γ are involved, the effect of crude culture supernatant (25% v/v of PWM-activated T cells) and of rIL-2 (50 U/ml) on the HLA class II determinants expression was analysed in cultures of monocytes cultured alone or with T cells in the presence and absence of PWM. It was found that neither IL-2 nor the crude supernatant had any effect on the above described changes in the expression of class II determinants (not shown). From these data we concluded that the reduction of some HLA class II antigens on gated monocytes is a phenomenon induced by PWM in the presence of T cells. Furthermore, our data indicate that this phenomenon is not mediated or prevented by a soluble factor, including IFN-γ and IL-2.

Proportion of CD14$^+$ cells in cultures of monocytes and T cells stimulated with PWM

The data already presented suggested that the expression of various class II determinants on monocytes is independently regulated in the presence of PWM-activated T lymphocytes. However, since we analysed gated cells, it could not be excluded that after culture some T lymphocytes may fit to the monocyte gate and be responsible for dilution of the monocytes. Therefore, the expression of a monocyte differentiation antigen (CD14) in this cell population was also measured. As shown in Fig. 3, in PWM-activated cultures of monocytes and T lymphocytes the proportion of cells which expressed monocyte differentiation antigen (CD14$^+$ cells) was dramatically reduced, although this was not seen in cultures of monocytes stimulated with PWM in the absence of T cells. The proportion of cells which co-expressed CD14 and HLA-DR determinants was also analysed at different time intervals in PWM-activated cultures of monocytes and T cells (not shown). The reduction of HLA-DR$^+$ correlated with the decrease of CD14$^+$ cells and was observed already after 24 hr. At the beginning of the culture 93% of CD14$^+$ cells were also HLA-DR$^+$; from 24 hr onwards all CD14-positive cells co-expressed HLA-DR. Since it is known

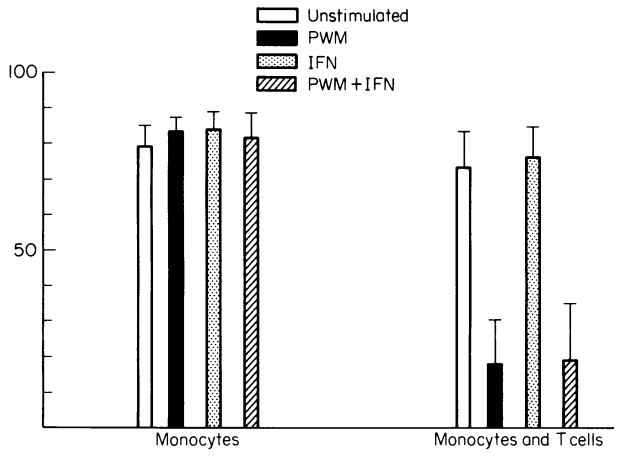

Figure 3. Presence of CD14-positive cells in cultures of monocytes and T cells. Monocytes were cultured alone or in the presence of T cells in numbers corresponding to those given in the legends to Figs 1 and 2. The results are from three separate experiments evaluated, using a pre-set analysis gate.

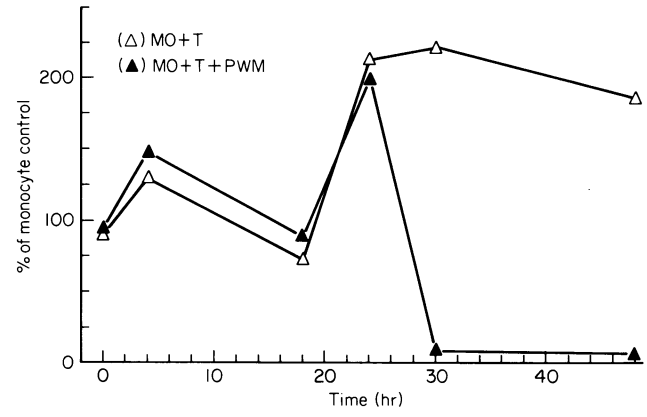

Figure 4. Latex-induced chemiluminescence. Monocytes, 1×10^4, were cultured with 4×10^4 T cells in chemiluminescence vials in the presence or absence of PWM. In parallel, as a control, the same numbers of monocytes were cultured without T cells in the presence of PWM. At indicated time intervals the response to latex stimulation was recorded. The results (1–10 min integral) are shown as percentage of CL response of monocytes cultured without T cells.

that monocyte differentiation antigens may be down-regulated by T-cell products (Firestein & Zvaifler, 1987; Jayaram *et al.*, 1989), cultures were also evaluated for the presence of cells containing non-specific esterase (NSE). The percentages of NSE$^+$ cells at 18 and 48 hr were 22·5% and 17·5%, respectively, in unstimulated, but 21·2% and 4·2% in PWM-activated cultures. Furthermore, the percentage of CD3$^+$ (Leu-4$^+$) cells in a population of gated monocytes in unstimulated 48-hr cultures of monocytes and T cells varied between 5% and 15%, while in PWM-activated cultures 65–85% of the cells were CD3$^+$. Moreover, double-fluorescence studies revealed that more than 90% of gated cells which expressed class II determinants were at the same time CD3$^+$. From this result we concluded that in PWM-activated cultures of monocytes and T cells, monocytes are most likely eliminated during the first 48 hr.

Chemiluminescence measurements

As indicated above, a significant reduction in the number of monocytes (CD14$^+$ cells) occurs within 48 hr in PWM-stimulated cultures, and the majority of cultured cells which express class II determinants are at that time T cells. This, however, does not exclude the possibility that a particular subset of monocytes which has lost CD14 antigen and class II determinants may still be present in the culture. In addition, any flow cytometry analysis and even detection of NSE$^+$ cells require harvesting of the culture, which may be subject to criticism. Therefore, as an alternative to tracing monocyte presence by phenotyping, we chose the measurement of latex-triggered luminol-dependent chemiluminescence. This method allows the detection of as few as 1×10^3 monocytes, and it is known that T cells do not generate chemiluminescence signals (Ernst *et al.*, 1984). Unstimulated or PWM-activated cultures of monocytes alone and mixed with T lymphocytes were set directly in tubes used for the chemiluminescence assay to avoid any possible cell loss. At 48 hr no chemiluminescence response could be generated in PWM-stimulated cultures of monocytes and T cells, although it was recorded in corresponding unstimulated cultures of monocytes and T cells and in PWM-activated cultures of monocytes alone (not shown). The activity of monocytes cultured with T cells in the presence of PWM was undetectable after approximately 30 hr of incubation (Fig. 4). In an additional experiment monocytes were mixed with T lymphocytes that had been preincubated with PWM for 24 hr. In this experiment the reduction of the chemiluminescence response had occurred already after 20 hr (not shown). We conclude that the reduction of monocyte chemiluminescence is due to their elimination and is mediated by PWM-activated T lymphocytes.

IFN-γ production in PWM-activated cultures

We have recently shown that PWM-induced IFN-γ production by T lymphocytes is monocyte-dependent and that as little as 1% of monocytes is sufficient to support the production of this lymphokine (J. Pryjma *et al.*, manuscript in preparation). Therefore, we used IFN-γ secretion as an additional assay to detect the monocytes. T cells alone or supplemented with 20% of monocytes were cultured for 72 hr (primary culture) in the presence or absence of PWM. Thereafter the culture supernatant was collected, and the cells were recultured in a fresh medium in the presence or absence of PWM (secondary culture).

In parallel, monocytes were also cultured in the presence or absence of PWM and were added (20%) to the secondary cultures. As shown in Table 1, the secretion of IFN-γ as measured in supernatants collected after 72 hr in primary cultures was dependent on the presence of monocytes. In secondary cultures, cells derived from unstimulated cultures of monocytes and T cells could be triggered to produce IFN-γ, whereas the same cells stimulated with lectin during the primary culture were only responsive when monocytes were added.

Expression of CD14 and HLA class II antigens in cultures stimulated with PWM in the absence of CD8 cells

To find out whether CD8$^+$ lymphocytes are involved in monocyte elimination, T cells were depleted of Leu-2a$^+$ subsets

Table 1. Secretion of IFN-γ in cultures of monocytes and T cells

Primary culture		Secondary culture	
Culture at Day 0	IFN-γ (U/ml) at Day 3	Addition at Day 3	IFN-γ (U/ml) at Day 6
T cells	<1	—	<1
		PWM	<1
		PWM + MO_1	131·6
		PWM + MO_2	166·0
T cells + PWM	<1	—	<1
		PWM	<1
		PWM + MO_1	86·0
		PWM + MO_2	88·2
T cells + MO	<1	—	<1
		PWM	69·8
		PWM + MO_1	117·8
		PWM + MO_2	199·2
T cells + MO + PWM	314·1	—	<1
		PWM	<1
		PWM + MO_1	27·8
		PWM + MO_2	34·6

After 3 days of culture cells were washed and recultured in a fresh medium in the presence or absence of PWM. To some cultures 20% of parallel cultured unstimulated (MO_1) monocytes or monocytes precultured for 48 hr with PWM (MO_2) were added.

Table 2. Expression of HLA-DR, HLA-DQ and CD14 antigen in PWM-stimulated cultures depleted of $CD8^+$ T lymphocytes*

Cells cultured	Mitogen	% of cells which express		
		CD14	HLA-DR	HLA-DQ
Monocytes + T cells	None	87·2	93·3	58·1
Monocytes + T cells	PWM	30·8	40·2	19·1
Monocytes + T ($CD8^-$) cells	None	83·4	91·2	63·0
Monocytes + T ($CD8^-$) cells	PWM	31·4	55·3	28·1

* Monocytes were mixed with T cells or T lymphocytes depleted of $CD8^+$ cells in a ratio of 1 : 4 and cultured for 48 hr before flow cytometry analysis. An analysis gate for monocytes was set according to forward and side scatter signals.

by panning and cultured with monocytes in the presence of PWM. As shown in Table 2, the depletion of $CD14^+$, HLA-DR and $HLA\text{-}DQ^+$ cells was comparable in the presence and absence of $CD8^+$ T lymphocytes, indicating that they are not responsible for monocyte elimination from the culture. From this experiment we conclude that the presence of $CD8^+$ cells in the culture is not essential for monocyte elimination in PWM-stimulated cultures.

DISCUSSION

These studies addressed the question of monocyte changes that occur in PWM-stimulated cultures of T cells and monocytes. The basic finding was an observation that monocytes are efficiently eliminated from such cultures by $CD4^+$ T lymphocytes. This conclusion was reached after flow cytometry analysis of cultured cells and functional studies.

By flow cytometry we followed the expression of class II determinants and monocyte differentiation antigen CD14. This approach was chosen, since IFN-γ, which is known to be released by activated T lymphocytes, induces the expression of class II determinants on monocytes (Gonwa *et al.*, 1986; Hoover & Meltzer, 1989) and down-regulates the expression of CD14 (Firestein & Zvaifler, 1987; Jayaram *et al.*, 1989). Our data clearly indicate that IFN-γ, when added to unstimulated or PWM-activated cultures of monocytes, can increase the expression of class II determinants on monocytes. Similarly, this lymphokine increased the expression of class II determinants when added to unstimulated cultures of monocytes and T cells. In contrast to recent observations of others (Firestein & Zvaifler, 1987) we did not see any significant changes in the expression of CD14 antigen after treatment of isolated monocytes with IFN-γ. The reason for this discrepancy remains unknown, but possible explanations are differences in experimental protocols and the origin of the monoclonal antibody used. PWM itself had no direct effect on HLA class II antigen expression on monocytes. However, when monocytes were cultured together with T lymphocytes and PWM, the proportion of cells expressing HLA-DR and DQ was reduced after 48 hr of culture. In the same cultures the reduced proportion of cells expressing the monocyte differentiation antigen (CD14) was observed. The above phenomenon was neither prevented nor augmented by IFN-γ, IL-2 or other soluble factors produced by PWM-activated T-lymphocytes, which indicates that the reduction in the proportion of $CD14^+$ cells and of cells expressing HLA-DR or DQ cells requires activated T cells and perhaps cell to cell contact. In our studies, we focused primarily on monocytes defined by their characteristic pattern of forward and side scatter during the analysis in the flow cytometer. Because of this approach our findings could be regarded as indicating: (i) the dilution of analysed monocytes by activated T cells with patterns of forward scatter to side scatter similar to monocyte scatter patterns; (ii) the loss of the CD14 molecule and some class II antigens from the monocyte surface; (iii) loss of monocytes. Indeed, the percentage of Leu-4^+ ($CD3^+$) cells in the analysis gate was substantially increased in samples taken from PWM-activated cultures, which indicates that the analysed population was diluted by T cells. However, this cannot be the only explanation, since the percentage of $CD14^+$ (Leu-$M3^+$) cells in the whole (ungated) population of cultured cells was also reduced. This reduction was parallel to the increased proportion of $CD3^+$ cells in PWM-activated cultures, which reached up to 95%, in contrast to unstimulated cultures where it never reached 90% (not shown). Furthermore, in PWM-activated cultures the proportion of cells with non-specific esterase activity was

decreasing with time. Therefore, our flow cytometry analysis data strongly suggest that monocytes are eliminated from mitogen-activated cultures.

Similar conclusions can be drawn from functional studies, namely the ability of monocytes to respond by chemiluminescence and to support IFN-γ production by T lymphocytes. In these functional tests cells were not transferred from the original culture vessels for analysis, which made the possibility unlikely that some monocytes were lost during sample handling.

By monitoring chemiluminescence response we could demonstrate that monocytes are eliminated after approximately 30 hr of culture with PWM-activated T cells, and that this time is shorter if lectin-preactivated T cells are added to monocytes.

Similarly, it was found that in PWM-stimulated cultures of monocytes and T lymphocytes no IFN-γ production can be triggered after 72 hr unless an additional number of monocytes is added. This indicates the absence of functionally active monocytes, although PWM-activated T cells can be triggered for the second time to produce IFN-γ, and monocytes cultured for 72 hr in the presence or absence of PWM are effective as accessory cells for the T-cell response measured by IFN-γ production.

In summary, we conclude that monocytes are eliminated from the culture by a mechanism which is triggered by PWM and requires T cells. Furthermore, our data suggest that monocyte elimination requires the presence of activated T cells as effectors, since: (i) IFN-γ, IL-2, or crude culture supernatants were not able to mimic their presence; (ii) PWM-preactivated T cells are more effective than non-activated T cells in the elimination of monocytes from the culture (as judged from chemiluminescence studies). Recently the appearance of cytotoxic CD4$^+$ T lymphocytes, natural killer (NK) and lymphokine-activated killer (LAK) cells, which are able to kill antigen-pulsed monocytes, has been observed by several investigators (Blanchard *et al.*, 1989; Hansen *et al.*, 1987; Ottenhoff *et al.*, 1988). Our preliminary data suggest that the depletion of CD8$^+$ cells from the culture does not prevent monocyte killing, indicating that CD4$^+$ lymphocytes may be responsible for this phenomenon. The percentage of Leu-11$^+$ cells was not increased in our culture (unpublished observation), which seems to indicate that NK cells do not play an important role in the described phenomenon. The disappearance of monocytes coincides with the appearance of CD3$^+$, HLA-DR and DP$^+$ lymphocytes. The presence of these cells is perhaps responsible for the relatively stable proportion of HLA-DP-positive cells in the monocyte gate. The expression of predominantly HLA-DR and DP, but not HLA-DQ, on T cells in PWM-activated cultures is similar to that described after T-cell activation with CD3 and other stimuli (Diedrichs & Schendel, 1989; Gansbacher & Zirr, 1988; Robbins *et al.*, 1988). It remains to be established whether HLA-DP is co-expressed with HLA-DR and whether these cells play any role in monocyte elimination (killing).

The biological significance of our findings is not clear at the moment. However, it seems to represent a more general phenomenon, as we also observed the reduction of CD14-positive cells in cultures activated by PHA or anti-CD3 antibody (unpublished data). Furthermore, our data may shed new light on the *in vitro* immunoregulatory role of monocytes by showing that any immunoregulatory effect of monocytes is limited to the beginning of the culture. We also wish to stress that although lymphokines, like IFN-γ, can profoundly change the function and phenotype of monocytes, monocytes may not be available for their action at the time of their release.

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