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Defective macrophage antigen presentation following haemorrhage is associated with the loss of MHC class II (Ia) antigens

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SUMMARY

Although recent studies indicate that severe and prolonged haemorrhage, despite adequate fluid resuscitation, induces profound depression of cell-mediated immunity, the mechanism of this remains unknown. Since macrophages (Mϕ) play a key role in the development of a competent immune response by the presentation of antigens, the study investigated (i) whether the capacity of the Mϕ to present antigen is altered even following mild hypotension, and (ii) what effects do different degrees of hypotension have on the Mϕ-mediated processes associated with antigen presentation (i.e. the expression Ia antigen, membrane-associated IL-1 or the secretion of IL-1). The results indicate that a minimal drop in blood pressure to ~ 50 mmHg (1 hr duration) was sufficient to depress Mϕ antigen presentation (AP). Similarly, even a transient hypotensive episode of 15 min duration at 35 mmHg was sufficient to produce a pronounced decline in AP. Decreased AP was observed as early as 12 hr after the haemorrhagic episode (35 mmHg; 1 hr) and remained detectable for at least 120 hr thereafter. The reductions in AP capacities were qualitatively similar in both peritoneal and splenic populations, and were not attributable to surgical stress, heparinization or ether anaesthesia. Determination of IL-1 production, as well as membrane-bound IL-1 levels, in these cell populations showed no significant difference from controls. However, a significant decrease was observed in the percentage of Ia antigen (MHC class II)-positive Mϕ, suggesting that reduced AP following haemorrhage may be related to the inability of these cells to express Ia.

INTRODUCTION

Studies examining traumatic injury due to burn and soft tissue damage in man clearly indicate profound suppression of both cell-mediated and humoral immuno-responsiveness in the host (Miller, Miller & Trunkey, 1982; Antonacci *et al*., 1984; Hamid *et al*., 1984). However, until recently little or no attention had been focused on the contribution of haemorrhage, a commonly encountered complication of traumatic and/or surgical injury, on the host's immuno-responsiveness. Studies by Stephan *et al*. (1987a, c) have demonstrated that splenocyte functions, such as proliferative responses, mixed lymphocyte reactions, IL-2 production, as well as natural killer cell activity, are depressed following simple haemorrhage, and persists despite adequate fluid resuscitation. Recent studies by Abraham & Freitas (1989) using a mouse model of hypotension (without resuscitation) not only confirmed these initial observations but have also demonstrated a reduction of the capacity of mouse splenocytes to express receptors for IL-2, as well as changes in splenocyte cell size, in the absence of any other marked phenotypic changes. Such a lack of immuno-responsiveness could, in part, account for the enhanced susceptibility to sepsis seen in mice following simple haemorrhage (Stephan *et al*., 1987a). Most recently, it has been reported (Stephan *et al*., 1989) that immediately following haemorrhage, macrophage (Mϕ) antigen presentation (AP) capacity was depressed. However, this study did not investigate whether transient or prolonged hypotension is required to produce such a defect. Moreover, little is known about the duration of the depression induced in Mϕ AP following a hypotensive episode. Since the Mϕ plays a key role in the generation of both cell-mediated and humoral immuno-responsiveness, it appears important to determine: (i) whether mild and or transient hypotension would also depresses Mϕ AP; and (ii) which Mϕ-mediated processes associated with AP are altered following haemorrhage and resuscitation. It has been proposed that at least two requirements must be met—first, that antigen is presented in a major histocompatibility complex (MHC) II (Ia)-restricted fashion; second, that the T helper cell requires a co-stimulatory signal (i.e. IL-1, etc.)—before competent Mϕ AP to the T-cell can take place (Unanue & Allen,

Abbreviations: AP, antigen presentation; BP, mean blood pressure; Con A, concanavalin A; FCS, fetal calf serum; IFN-γ, interferon-gamma; IL-1, interleukin 1; mIL-1, membrane-associated interleukin-1; LPS, lipopolysaccharide; Mϕ, macrophage(s); PBS, phosphate-buffered saline.

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1987). In view of this, it was attempted to determine not only the capacity of Mϕ to present antigen following haemorrhage and resuscitation, but also to examine their capacity to express Ia antigen, membrane IL-1 (mIL-1) as well as their ability to produce IL-1.

MATERIALS AND METHODS

Animals

Inbred C3H/HeN (Charles River Labs, Portage, MI) male mice (6–8 weeks old), weighing 20–25 g, were utilized.

Haemorrhage model

Haemorrhage was induced in the mouse according to the methods of Stephan *et al.* (1987a). In brief, mice were lightly anaesthetized with ether and restrained in the supine position. Both femoral arteries were then catheterized, under aseptic conditions, with number 10 polyethylene tubing using a minimal dissection technique. The animal was then heparinized (2 U beef lung heparin/10 g body weight; Upjohn Labs, Kalamazoo, MI) and allowed to awaken. Blood pressure was continuously monitored by attaching one of the catheters to a strain gauge pressure transducer (Model P23 ID, Gould Inc., Oxnard, CA) coupled to a polygraph (Model 7D, Grass Inst., Quincy, MA). Upon awakening the animal was bled through the other catheter to a mean blood pressure (BP) of 70, 50, or 35 ± 5 mmHg (normal BP prior to haemorrhage was $94{\cdot}4 \pm 8{\cdot}3$ mmHg), which was maintained for 15, 30 or 60 min. In an effort to minimize the loss of adherent cells, the blood was extracted and stored in a polypropylene syringe during this period. At the end of the hypotensive period the animal's own shed blood was returned, followed by the infusion of Ringer's lactate (twice the shed blood volume) to provide adequate fluid resuscitation. The catheters were then removed, the vessel ligated and the groin incisions closed. There was no mortality with this model irrespective of the duration or the severity of hypotension induced. Control animals underwent the same surgical procedures, which included ligation of both femoral arteries, but haemorrhage was not induced.

Preparation of macrophage populations

For a given experimental group, two animals were pooled following killing and peritoneal lavages were performed as described by Wirth & Kierzenbaum (1984) to obtain resident peritoneal Mϕ. Cells were washed once in phosphate-buffered saline (PBS) by centrifugation (280 ***g***, 15 min, 4°) and resuspended at a concentration of 1×10^6 viable cells/ml of Click's medium (Irvine Sci., Santa Ana, CA) (cell viability was determined by trypan blue exclusion). Five millilitres of this suspension were then plated onto 60-mm diameter plastic petridishes and incubated for 2 hr (37°, 5% CO_2, 90% humidity). Non-adherent cells were removed by repeated washing with Click's medium. This protocol provides adherent cells that are greater than 99% positive by non-specific esterase staining and exhibit typical Mϕ morphology.

Splenic Mϕ were prepared according to the methods previously described by Kaye *et al.* (1983) and as utilized in this laboratory (Stephan *et al.*, 1989).

It should be noted that no significant differences were observed in the viability (>95% by trypan blue exclusion) or yields in the number of cells extracted from either peritoneum or spleen following haemorrhage compared to shams.

D10.G4.1 T-helper cell clone

The mouse D10.G4.1 T helper 2 cell clone (a gift from Dr C. A. Janeway, Yale University, New Haven, CT) was maintained as described by Kaye *et al.* (1983).

Antigen presentation assay

The capacity of either peritoneal or splenic Mϕ to present antigen to D10.G4.1 cloned helper T cells was carried out according to methods of Kaye *et al.* (1983). In brief, the monolayer of adherent cells was incubated for 30 min (37°, 5% CO_2, in the dark) with 50 μg mitomycin C/ml PBS (Sigma Chem. Co., St Louis, MO). The plates were then washed three to four times with PBS, overlaid with 1·5 ml Click's media plus 10% heat-inactivated fetal calf serum (FCS; Sigma Chemical Co., St Louis, MO) and gently scraped off the surface with the aid of rubber policeman. Cells were centrifuged, viability determined and concentration adjusted to 3×10^5 cells/ml of Click's media with 10% FCS. The adherent cells were transferred in 50-μl aliquots to wells of 96-well microtitre plates and serially diluted so as to obtain cell concentrations ranging from 27,000 to 300 cells/well. A 50-μl aliquot of D10.G4.1 cells (2×10^4 cells/ml) was added to each well. This was followed by 50 μl of conalbumin (300 μg/ml; Sigma Chem Co.), the specific antigen to which the D10.G4.1 cell line has been sensitized (Kaye *et al.*, 1983). A duplicate dilutions series using the same cells was also set-up but the 50 μl of conalbumin were replaced with media alone, thus providing a non-specific antigen control. The cultures were incubated for 48 hr at 37° with 5% CO_2. At the end of this time period, 1 μCi of [^{3}H]thymidine ([^{3}H]TdR) was added to each well (specific activity 6·7 Ci/mmol; New England Nuclear, Wilmington, DE) and cultures were incubated for a further 14–16 hr. The cultures were then harvested with a multiple automated sample harvester (Skatron AS, Tranby, Norway) onto a glass-fiber filter mat and counted in a liquid scintillation counter (model 1205 Betaplate, Pharmacia/LKB Nuclear, Gaithersburg, MD). The extent of antigen-specific incorporation of [^{3}H]TdR was determined by subtracting the radioactivity incorporated in the paired non-specific antigen control (in the absence of conalbumin).

Membrane and secreted IL-1 assay

Membrane-bound or secreted IL-1 activity was determined using the D10.G4.1 cell line, which responds to either form of IL-1 in a dose-dependent fashion in the presence of concanavalin A (Con A; Pharmacia/LKB Biotech. Inc., Piscataway, NJ) (Kaye *et al.*, 1984). The method for determining the amount of Mϕ membrane-bound IL-1 activity using D10.G4.1 cells is essentially the same as that outlined by Stephan *et al.* (1987b), in which 50 μl of Con A, a non-specific T-cell mitogen, in 10 μg/ml Click's medium with 10% FCS concentration, were substituted for the specific antigen (conalbumin). It should be noted that no significant proliferation was observed when Con A or IL-1 were added alone.

The capacity of mouse Mϕ to produce IL-1 was assessed by determining the amount of IL-1 secreted by these Mϕ at 1×10^6 cells/ml/well of a 24-well microculture plate, upon stimulation with 10 μg of lipopolysaccharide W (LPS; from *Escherichia coli* 055:B5; Difco Labs, Detroit MI)/ml Click's medium with 10% FCS for 24 hr (37°, 5% CO_2). The cell supernatants were collected, filtered, aliquoted and stored at −70° until assayed.

The IL-1 activity in these culture supernatants was determined by adding serial dilutions of the supernatant (as opposed to varying the number of Mϕ, as in the mIL-1 or AP assays) to the D10.G4.1 cells (2×10^4 cells/well), in the presence of Con A. Proliferation of the D10.G4.1 was measured as described above. All sample or cell dilutions used in the assays above were tested in triplicate.

The unit(s) of IL-1 activity per ml or per 3×10^5 adherent cells was determined by comparison of the curves produced from dilution of the unknowns to that generated by dilution of a purified human IL-1 standard (5 U/ml; Genzyme, Boston, MA), according to the methods of Mizel (1981).

Determination of the percentage Ia-positive cells

Monolayers of either peritoneal or splenic Mϕ were prepared in triplicate for each experimental group on 3-mm diameter glass wells of otherwise Teflon-coated microscope slides (Cel-line, Newfield, NJ), according to the methods of Ayala & Kierszenbaum (1987). After removal of the non-adherent cells, the cultures were either immediately fixed in 1% paraformaldehyde (Sigma Chem. Co.) in PBS at 4° or incubated for 24 hr with conditioned medium (see below) or 100 U recombinant mouse interferon-gamma (IFN-γ; Amgene, Thousand Oaks, CA)/ml for 24 hr at 37°, 5% CO_2 and then fixed as described above. The fixed slides were washed three times (10 min, at 24°) in PBS prior to incubation with 10 μg mouse IgG/ml (Sigma Chem. Co.) for 30 min at 24°. The slides were again washed three times in PBS, after which they were stained for 60 min at 37° with fluorescein-conjugated mouse Ia^k alloantisera (Accurate Scientific, Westbury, NY). Residual antibody was removed by three more washings in PBS, following which the slides were dried and stored in the dark at 4° prior to examination. No fewer than 200 cells were screened in each culture, recording both the number of fluorescent cells (Ia-positive) and non-fluorescing cells. From these values, the percentage of Ia-positive cells was then calculated. All samples were tested in triplicate.

Splenocyte-conditioned media

The spleens from an untreated mouse were removed and then gently ground between two frosted microscope slides so as to produce a single cell suspension. Following hypotonic lysis of the erythrocytes, the unfractionated cell suspension was washed twice by centrifugation, at 300 *g* for 10 min at 24°, and resuspended in enough Click's media containing 10% FCS to yield a final concentration of 1×10^6 cells/ml. The splenocytes were then incubated for 24 hr (37°, 5% CO_2) in the presence of 10 μg Con A. At the end of this period the suspension was centrifuged (3,500 *g*, 20 min, 24°), the supernatant saved and the pellet discarded. Residual Con A activity in the supernatant was inactivated by the addition 20 mg alpha-methyl-D-mannopyranoside/ml (Sigma Chem. Co.). The supernatants were then filter-sterilized, aliquoted, and stored at −70° until used.

Presentation of data and statistical analysis

The data are presented as a mean and standard error of three or more separate experiments under each condition. Differences in the experimental means were considered to be significant if $P < 0.05$, as determined by the Mann-Whitney *U*-test.

RESULTS

Effects of haemorrhage on macrophage antigen presentation

The depressed capacity of peritoneal Mϕ, harvested 24 hr post-haemorrhage (1 hr duration, BP ~35 mmHg), to present antigen compared to Mϕ taken from either sham-control animals or from surgically unmanipulated animals, is shown in Fig. 1. The dose–response nature of this assay is illustrated by the dependence of D10.G4.1 proliferation ([^{3}H]TdR incorporation) on the number of Mϕ present. Further examination of Fig. 1 demonstrates that the alteration in the capacity of peritoneal Mϕ to present antigen is also not readily attributable to surgical stress. While not shown, similar studies indicated that heparinization of the animal *per se* was not responsible for the depressive effects seen following haemorrhage. For the sake of clarity, throughout the remainder of this manuscript the effects of a given experimental treatment on Mϕ AP are illustrated as a percentage of the capacity of the respective sham-control to present antigen, instead of antigen-specific [^{3}H]TdR incorporation.

Effects of different levels and durations of hypotension on macrophage antigen presentation

The results presented in Fig. 2 illustrate the effect of BP on the capacity of both peritoneal and splenic Mϕ harvested 24 hr post-haemorrhage to present antigen. BP had to be decreased and maintained for 1 hr below 70 mmHg BP before significant depressive effects were seen in the capacity of Mϕ to present antigen. The results of experiments in which length of the hypovolemic episode was varied (BP 35 mmHg, cells harvested 24 hr post-haemorrhage) indicate that as little as 15 min of hypotension is sufficient to produce a significant decline in the capacities of both splenic adherent as well as peritoneal Mϕ to present antigen (Fig. 3). Increasing the duration of haemorrhage to 30 or 60 min had no further depressive effect.

AP in both cell populations appeared markedly depressed as early as 12 hr after the haemorrhagic episode (BP 35 mmHg; 1 hr duration) (Fig. 4). One-hundred and sixty-eight hours were required before this function returned to normal.

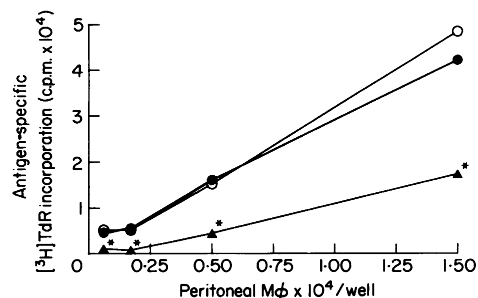

Figure 1. The effect of haemorrhage (1 hr duration, 35 mmHg) on antigen presentation by peritoneal macrophage harvested to present antigen (conalbumin) to D10.G4.1 cells. Macrophage antigen presentation was determined as described in the Materials and Methods. The data are presented as the mean (triplicate determination) antigen-specific incorporation of [^{3}H]TdR induced by peritoneal macrophage harvested from untreated (○), sham-control (i.e. sham catheterization) (●) or 24 hr post-haemorrhage (▲) C3H/HeN mice. Differences where significant ($P < 0.05$) are indicated by *.

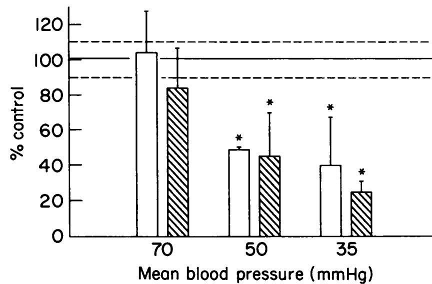

Figure 2. The effects of haemorrhage (1 hr duration) maintained at various fixed mean blood pressures (70, 50 or 35 mmHg) on the capacity of peritoneal macrophage (□) or splenic macrophage (■) (cells harvested 24 hr post-haemorrhage) to present conalbumin to D10.G4.1 cells. The protocols for antigen presentation were as outlined in the Materials and Methods. The data are presented as a mean percentage ± SEM of the paired sham-control capacity to present antigen when the concentration of macrophage is 5000/well. The dashed lines represent the variation (SEM) present in the sham-treated groups. Where the experimental groups differed significantly ($P < 0.05$) it is indicated by *.

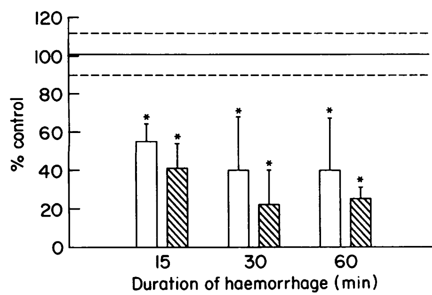

Figure 3. The suppressive effects of varying periods (15, 30 or 60 min) of haemorrhage (35 mmHg) on the capacity of peritoneal macrophage (□) or splenic macrophage (■) (cells harvested 24 hr post-haemorrhage) to present conalbumin to D10.G4.1 cells. The protocols for antigen presentation were as outlined in the Materials and Methods. The data are presented as the mean percentage ± SEM of the paired sham-control capacity to present antigen when the concentration of macrophage is 5000/well. The dashed lines represent the variation (SEM) present in the sham-treated groups. Where the experimental groups differed significantly ($P < 0.05$) it is indicated by *.

Expression of mIL-1 and IL-1 production

Mϕ which were harvested at either 12 or 24 hr post-haemorrhage (1 hr duration, 35 mmHg) showed no significant decline in the expression of mIL-1 (Table 1). In fact, a slight increase in mIL-1 expression is present in the splenic Mϕ population 12 hr post-haemorrhage. Similarly, no significant difference was observed in the capacity of peritoneal Mϕ harvested at either 12 or 24 hr post-haemorrhage to produce IL-1 compared to the sham controls (data not shown).

Ia antigen expression

The percentage of Ia-positive cells present 24 hr post-haemorrhage (1 hr duration, 35 mmHg) was significantly decreased in both peritoneal and splenic populations (Fig. 5). In additional studies, peritoneal Mϕ were co-cultured for 24 hr with conditioned medium from mouse splenocytes prior to determination of the percentage Ia-positive cells. Such supernatants have been shown to contain IFN-γ (Leonard, Rucu & Meltzer, 1978),

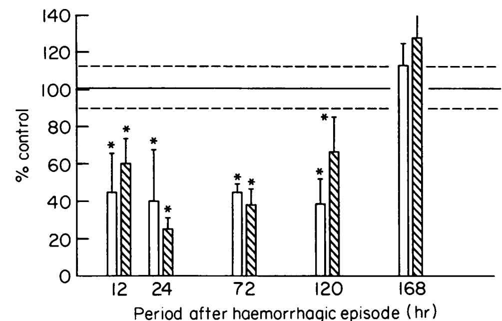

Figure 4. The duration of the suppression of the capacity of peritoneal macrophage (□) or splenic macrophage (■) to present conalbumin to D10.G4.1 cells when these cells were harvested at varying periods of time following haemorrhage (1 hr duration, 35 mmHg). The protocols for antigen presentation were as outlined in the Materials and Methods. The data are presented as a mean percentage ± SEM of the paired sham-control capacity to present antigen when the concentration of macrophage is 5000/well. The dashed lines represent the variation (SEM) present in the sham-treated groups. Where the experimental groups differed significantly ($P < 0.05$) it is indicated by *.

Table 1. Membrane IL-1 activity of peritoneal and splenic Mϕ taken from mice at varying periods of time after haemorrhage*

Exp.	Treatment group	mIL-1 activity (U/3 × 10^5 cells) Peritoneal Mϕ	Splenic Mϕ
1	12 hr post-haemorrhage		
	Experimental	9·0 ± 0·6	4·5 ± 0·4†
	Control	6·8 ± 1·6	3·7 ± 0·3
2	24 hr post-haemorrhage		
	Experimental	14·2 ± 2·4	1·3 ± 0·1
	Control	14·1 ± 3·2	0·9 ± 0·4

* The mice were bled and maintained at a mean blood pressure of 35 mmHg for 60 min. mIL-1 levels were determined as described in the Materials and Methods.

† $P < 0.01$ compared to control.

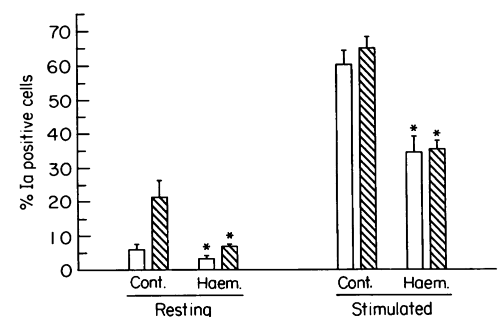

Figure 5. Haemorrhage (1 hr duration, 35 mmHg)-induced reduction in the percentage Ia-positive peritoneal macrophage (□) or splenic macrophage (■) which were either resting or stimulated prior to staining with FITC conjugate mouse Ia^k alloantisera. The data are presented as the mean percentage Ia-positive cells ± SEM; where the experimental differed significantly ($P < 0.05$) from the control it is indicated by *.

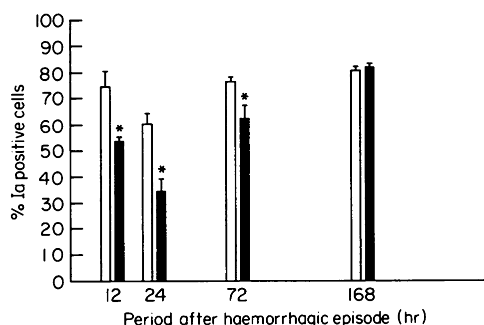

Figure 6. Haemorrhage-induced reduction in the percentage Ia-positive activated peritoneal macrophage when cells were harvested at differing periods of time following the haemorrhagic episode (1 hr duration, 35 mmHg). The data are presented as the mean percentage Ia positive cells ± SEM; where the experimental (■) differed significantly ($P < 0.05$) from the control (□) it is indicated by *.

which up-regulates Mϕ Ia expression (Hamilton & Adams, 1987). It can be seen that cells treated with this supernatant show a marked increase in the percentage Ia-positive cells for both haemorrhage and sham-control populations (Fig. 5). However, a significant decrease in the percentage of Ia-positive cells is still apparent in those cells taken from animals 24 hr post-haemorrhage. Additionally, if IFN-γ was substituted for splenocyte-conditioned media, it resulted in enhancement but not restoration of Mϕ Ia expression (24 hr post-haemorrhage, 18·9 ± 2·7% versus control, 50·0 ± 3·0% Ia-positive peritoneal Mϕ). Figure 6 illustrates that the depression of Ia antigen expression remains significant through the first 72 hr following the haemorrhagic episode, but is not observable 168 hr post-haemorrhage.

DISCUSSION

The results presented above not only confirm the earlier observation that Mϕ AP is depressed immediately following haemorrhage (Stephan *et al.*, 1989), but also show that even a transient hypotensive episode is sufficient to produce a marked depression in both splenic and peritoneal Mϕ functions, and that this defect persists despite adequate fluid resuscitation. In light of the previous studies (Chaudry *et al.*, 1989; Stephan *et al.*, 1987a,c) in which it was demonstrated that splenocyte functions, such as proliferative responses, mixed lymphocyte reactions, IL-2 production, as well as natural killer cell activity, were all depressed following haemorrhage, it could be concluded that the effects of haemorrhage on the immune system are systemic in nature.

It could be argued that the reason Mϕ AP function is decreased is due to a loss of Mϕ during or following haemorrhage. This, however, does not appear possible since in these studies haemorrhage *per se* did not induce any overt changes in the Mϕ yields or viability of cells taken from either the peritoneum or the spleen. Furthermore, studies by Abraham & Freitas (1989) also showed no significant change in the proportions of the various lymphoid cell types resident in organs such as the spleen or lymph nodes following haemorrhage. It remains to be determined whether or not the actual make-up of these populations [i.e. mature versus resting (as well as percentage dentritic cell types in the spleen) Mϕ in these populations] are altered following haemorrhage.

Another possibility is that the suppression of Mϕ AP is simply due to heparinization or surgical stress on the animal. Neither of these possibilities would appear to be valid since no depressive effects on AP capacity were observed when either of these factors were assessed alone (Fig. 1). In addition, previous studies at the Michigan State University indicate that retransfusion of heparinized syngeneic whole blood is not the stimulus to depress Mϕ AP (Stephan *et al.*, 1988).

While the results provided above further establish and clarify the extent of the dysfunction associated with Mϕ AP, they provide few clues as to the cause of this defect. The second objective of this study was therefore directed towards defining the effects of haemorrhage on those processes associated with competent Mϕ AP. The ability of the Mϕ to present antigen to T helper lymphocytes is controlled by two major features: (i) the processing and presentation of antigen in an MHC class II (Ia)-restricted fashion, and (ii) the elaboration of and/or the presence of IL-1 on the antigen-presenting cells' surface (Unanue & Allen, 1987; Kurt-Jones, Virgin & Unanue, 1985).

With this in mind, the initial studies focused on determining whether or not Mϕ from haemorrhaged animals exhibited any marked depression in IL-1 production or expression of mIL-1. There was no appreciable decline in the level of secreted or mIL-1 expression by Mϕ from haemorrhaged animals (Table 1). However, this does not rule out the possibility that changes in Mϕ production of other cytokines, such as IL-6 and tumour necrosis factor-alpha (TNF), which have also been reported to have some of the same co-stimulatory effects on T-cell proliferation as IL-1 (Garman *et al.*, 1987; Ranges *et al.*, 1989), are altered following haemorrhage. In this regard recent studies by Mizutani *et al.* (1989) indicate that while IL-6 in the presence of Con A and IL-1 has a small augmentative capacity, IL-6 by itself has little stimulatory effect on antigen-specific D10.G4.1 proliferation. In reference to TNF production by these Mϕ, studies at the Michigan State University show no significant decline in the capacity of peritoneal Mϕ to produce this cytokine when cells were harvested from mice at either 2 or 24 hr post-haemorrhage (Ayala *et al.*, 1990). These findings suggest that while these cytokines may be present in the Mϕ supernatants which were examined here, their relative contribution to D10.G4.1 proliferation would be minimal in comparison to IL-1. Finally regardless of whether these or other cytokines produce or expressed by Mϕ can also act as proliferative signals for the D10.G4.1 cell line, the fact remains that cells taken from haemorrhaged animals show no decline in their capacities to elaborate cytokines capable of inducing D10.G4.1 proliferation compared with the controls. Thus, the depression in AP observed following haemorrhage cannot be attributed to changes of these cells to either produce and/or express co-stimulatory cytokines, such as IL-1 or mIL-1.

Since IL-1 production as well as membrane-bound IL-1 expression were not altered, the Ia expression by these Mϕ following haemorrhage was studied. The results indicated that 24 hr post-haemorrhage there was a significant decrease in the percentage Ia-positive cells in both resting peritoneal and splenic Mϕ cell popuations (Fig. 5). Since there was no loss in either the number or viability of Mϕ obtained from the peritoneum or the spleen after haemorrhage, this suggests that the loss of Ia positive cells is due to a reduction of the overall Ia antigen expression by these cells.

To determine whether the defect in Ia expression could be

corrected, Mϕ were incubated *in vitro* with mouse splenocyte-conditioned medium. Conditioned media of this type is known to contain a variety lympokines, among which is IFN-γ (a well known Mϕ-activating factor that up-regulates Ia expression; Hamilton & Adams, 1987). Such treatment allowed not only the difference in the percentage of cells expressing Ia but also the capacity of these cells to undergo activation leading to enhanced Ia expression to be examined. While activation of these cells markedly increased the percentage Ia-positive cells in both haemorrhage and sham-control populations (Fig. 5), the significant decreases in the percentage of Ia-positive cells observed with resting cells following haemorrhage still persisted despite activation. It should be noted that this phenomena was observed even when recombinant mouse IFN-γ was substituted for splenocyte-conditioned media. Taken together, these results suggest not only a reduction of those cells expressing Ia, but that the capacity to maximally activate these cells may also be compromised following haemorrhage.

How Ia antigen expression is depressed following haemorrhage is unknown at this time. Livingston *et al.* (1988a) have reported that following trauma, IFN-γ production by human peripheral blood lymphocytes is suppressed. The inability of these cells to produce this mediator might account for the lack of MHC class II antigen expression in trauma patients (Polk *et al.*, 1986). In this vain, work by Livingston & Malangoni (1988) indicates that the capacity of rat's to ward off infection following haemorrhagic shock is enhanced by combined IFN-γ antibiotic therapy. It is worth noting that studies by Chaudry *et al.* (1989) and Abraham & Freitas (1989) showed that following haemorrhage mouse splenocytes exhibit a reduced ability to produce IL-2. Since this lymphokine is thought to have a regulatory role with regards to the production of IFN-γ (Farrar, Birchenall-Sparks & Young, 1986; Meidema *et al.*, 1985), it is tempting to speculate that mouse lymphocyte production of this mediator may also be depressed following haemorrhage. Work by Abraham & Freitas (1989), however, indicated that the capacity of mouse-activated splenocytes to produce IFN-γ is enhanced following haemorrhage. However, since their model utilized a fixed volume bleed-out without resuscitation, and as BP values were not reported, it is not known whether there was any significant decrease in BP in that model. Furthermore, since their model (Abraham & Freitas, 1989) differs significantly from those used in the majority of haemorrhage studies (Chaudry *et al.*, 1989; Donohoe *et al.*, 1986; Livingston *et al.*, 1988b), as well as the model utilized here, it remains to be determined whether IFN-γ production following haemorrhage and resuscitation is significantly altered. None-the-less, studies at the Michigan State University examining the administration of various immunomodulators following haemorrhage suggest that IFN-γ can partially restore antigen presentation capability of these Mϕ (W. Ertel, M. H. Morrison, A. Ayala, M. M. Perrin and I. H. Chandry, unpublished data).

A number of studies suggest that mediators such as prostaglandins, glucocorticoids, aldosterone, endotoxin, interferons alpha and beta, etc., can down-regulate Mϕ Ia antigen expression (Snyder, Beller & Unanue, 1982; Yem & Darmely, 1981; Steeg, Johnson & Oppenheim, 1989; Warren & Vogel, 1985; Ling, Warren & Vogel, 1985). With regards to IFN-γ, it is also known that several of the afore mentioned agents can also inhibit its production (Zlotnik *et al.*, 1985; Gemsa, 1981). Studies also indicate (Zlotnik *et al.*, 1985; Chaudry *et al.*, 1989) that prostaglandins can inhibit Mϕ AP. Since the levels of prostaglandins in the blood of haemorrhaged animals are increased (Lefer, 1983), it is possible that prostaglandins might be responsible for down-regulation of Ia expression in these cells. However, here again, it is unknown whether or not the levels of prostaglandins in mouse blood following haemorrhage are elevated. The same can be said of other down-regulators of MHC class II antigen expression, such as glucocorticoids, aldosterone, endotoxin, interferons alpha and/or beta, etc., as little is known concerning either the blood level of these agents or the capacity of various cell types to produce them following haemorrhage in mice.

While the precise mechanism of the depression of Mϕ AP is unknown, the results presented here clearly imply that simple haemorrhage, despite adequate resuscitation, produces a marked and prolonged depression in this function. In addition, the results indicate that severe hypotension, even for a short period of time, or a decrease of BP to approximately 50% of normal is sufficient to produce marked alterations in Mϕ AP. The inability of these Mϕ to present antigen following haemorrhage is associated with the loss of MHC class II (Ia) antigens. Since Mϕ function is depressed following trauma, it could be concluded that if significant blood loss is coupled with tissue injury then a more pronounced depression Mϕ function would occur.

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