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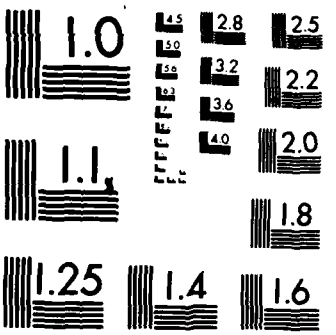

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IMMUNE DYSFUNCTIONS AND ABROGATION OF THE INFLAMMATORY RESPONSE BY ENVIRONMENTAL CHEMICALS

Richard G. Olsen
Department of Veterinary Pathobiology

For the Period
July 1, 1982 - June 30, 1983

Department of the Air Force
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Exposure of immune cells to UDMH induces immune cell dysfunction suggestive of impairment of the immunoregulatory component of the immune system. We found that UDMH does not alter the suppressor cell/helper cell ratio nor does it overtly alter the Ia receptors on immune cells. UDMH does significantly change prostaglandin E_2 synthesis. This chemical mediator is known to play a keynote in regulating immune cells.

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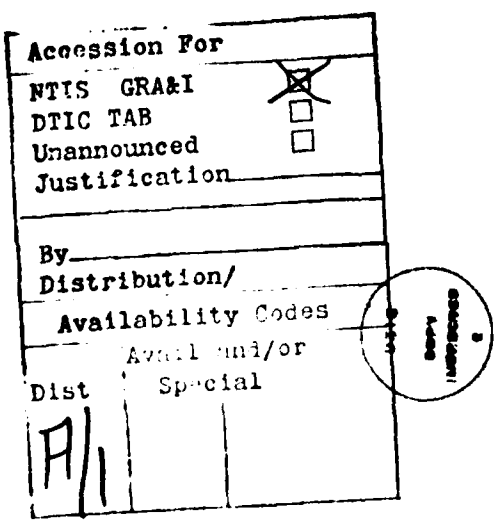

Progress Report

I. Research Objectives

Our efforts during FY 1982-83 have mainly been directed at characterization of the effects of 1,1-dimethylhydrazine (UDMH) on lymphocyte membrane function and antigen expression, and on prostaglandin E_2 (PEG_2) production by macrophages. These studies evolved in an attempt to elucidate the mechanism(s) by which UDMH causes immunoenhancement, which was observed in earlier studies (Tarr, M.J. et al., 1982). Specific objectives during the past year included:

A. Determine the effects of UDMH on immune cell subsets, determined by expression of Lyt 1.2, Lyt 2.2, and Thy 1.2 antigens on the cell surface of T-lymphocytes.

B. Determine the effects of UDMH on capping of lymphocyte concanavalin A (con A) receptors.

C. Determine the effects of UDMH on membrane Ia antigen expression by spleen cells.

D. Determine the effects of UDMH on PGE_2 production by adherent spleen cells and peritoneal macrophages.

E. Determine the effects of UDMH on intracellular cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in spleen cells.

F. Begin determination of the effects of UDMH on autologous mixed lymphocyte reaction.

G. Begin determination of the effects of UDMH on interleukin 1 (IL1) and interleukin 2 (IL2) production.

II. Status of Research

A. Effects of UDMH on immune cell subsets:

1. Background and methodology

Different functional subsets of murine lymphocytes can be distinguished by their cell surface antigens. Specifically, murine T-lymphocytes express a "Thy 1" antigen on their cell surface. The -lymphocytes can be further divided into helper cells which have "Lyt 1" antigens, and suppressor/cytotoxic cells which have "Lyt 2,3" antigens. In many instances disorders of the immune system have been associated with altered absolute numbers or ratios of these cell subsets.

Monoclonal rat antibodies directed against these murine antigens and conjugated with fluorescein thiocyanate (FITC) are commercially available and allow visualization and enumeration of lymphocytes expressing each antigen. In our experiments normal spleen cells were incubated in medium

(control) or medium containing 5, 10, 25, 50, 75, 100 or 200 µg/ml UDMH for 1, 2, 4 or 24 hrs. The cell suspensions were then washed, incubated with the FITC reagent, and viewed through an ultraviolet microscope. The percent of cells expressing each antigen was determined.

2. Results

a. Thy 1.2 antigen. Incubation with UDMH did not have any effect on the expression of Thy 1.2 antigen by spleen cells for the time periods tested (Table 1).

b. Lyt antigens. Spleen cells incubated for two hrs with UDMH showed a higher percent expression of Lyt 1.2 at all concentrations tested (Table 2). One hr and four hr exposures did not affect Lyt 1.2 expression. Lyt 2.2 expression was not altered by UDMH at any of the time periods tested.

3. Discussion

Based on these experiments, it does not appear that UDMH affects expression of lymphocyte subset antigens, except one antigen (Lyt 1.2) at one time period. The significance of this result is questionable since the control value was unusually low.

The results of these experiments may or may not be accurate, since the methodology (visualization of membrane fluorescence) does not permit one to quantitate the number of receptors, and allows only a subjective assessment of the fluorescence. The use of a fluorescence-activated cell analyzer would permit more definitive results.

B. Effects of UDMH on lymphocyte capping phenomenon

1. Background and methodology

"Capping" of mitogen or antigen by lymphocytes is a prerequisite to blast transformation and proliferation. The mitogen or antigen attaches to lymphocyte membrane receptors which then migrate to one location on the membrane before being internalized and processed. This can be visualized by using FITC-conjugated mitogen or antigen and visualizing the cells after a brief incubation period.

In these experiments, normal splenic lymphocytes were incubated with UDMH at 0 (control), 5, 10, 25, 50, 75, 100 or 200 µg/ml for 45 min., 2, 4, 24 or 48 hrs. FITC-conjugated concanavalin A was used for the last 45 minutes of the incubation period. They were then viewed through an ultraviolet microscope to determine the percentage of capped cells.

2. Results

UDMH did not affect the percent capping except after a 24 hr incubation with 25-200 µg/ml, which resulted in decreased capping percentages (Table 3).

3. Discussion

The significance of the decreased capping percentage after 24 hr exposure to UDMH is questionable since a) both shorter and longer incubation periods did not affect capping; and b) alterations in the in vitro immune function assays noted previously occurred after only 2 hrs incubation with UDMH. In addition, one would expect, if anything, a suppressed immune response with decreased capping, not an enhanced response such as we observed, and the decrease would have to occur much earlier than 24 hrs since it is one of the initial events in the immune response. Thus, we concluded that UDMH does not exert its immunoenhancement effects by altering capping or receptor mobility.

C. Effects of UDMH on membrane Ia antigen expression

1. Background and methodology

It is now well established that the "Ia" antigen is coded for by one of the immune response genes, and is expressed on the cell membrane of most macrophages and B-cells, and some T-cells (suppressor, some effector), as well as certain other cell types. Helper T-cells have a receptor for the Ia antigen. An optimum immune response requires interaction between the Ia antigen on the presenting macrophage and the Ia receptor on the T helper cell, and histocompatible identity among the participating immunocytes.

We examined the effects of UDMH on Ia antigen expression by plastic-adherent spleen cells (comprised mostly of macrophages and B-cells). This was accomplished using an indirect immunofluorescent assay, with monoclonal rat anti-mouse Ia followed by FITC-conjugated rabbit anti-rat immunoglobulin. Adherent splenocytes were incubated with 0 (control), 5, 10, 50 or 100 ug/ml UDMH for 2 hrs, then washed and further incubated for 24, 48, 72 or 96 hrs. After each incubation period the percent of cells expressing Ia antigen was determined by viewing through an ultraviolet microscope.

2. Results

UDMH did cause mild but significant increases in percent of cells expressing Ia antigen at various concentrations and times after exposure (Table 4). A good concentration effect was not observed, but increases were noted at 5 ug/ml for the 24 and 72 hr incubation periods; 10 µg/ml for the 48 and 72 hr incubation periods; and 100 µg/ml for the 24 and 48 hr incubation periods.

3. Discussion

Since an optimum immune response depends on Ia antigen expression by macrophages as a prerequisite to Ia antigen-receptor interaction with the helper T-cell, enhanced Ia antigen expression could theoretically contribute to an enhanced immune response. The UDMH could be causing this effect directly or indirectly through its effect on reducing PGE_2 synthesis (see below). Also, the effects of UDMH were slight and erratic,[2] which may again

reflect the methodology employed (see comments under A.3). If a fluorescent-activated cell analyzer becomes available, these experiments will be repeated.

D. Effects of UDMH on prostaglandin E_2 (PGE_2) production by adherent spleen cells and peritoneal macrophages

1. Background and methodology

One mechanism of regulation of immune responses has been shown to be by certain prostaglandins, particularly of the E series (PGE_1, PGE_2). PGE_2 alters the cyclic adenyl monophosphate: cyclic guanidyl monophosphate ratio in many cell types including lymphocytes which results in decreased lymphocyte activation and proliferation. The main source of PGE_1 or PGE_2 among immunocytes is newly activated macrophages. In the following experiments PGE_2 release by adherent splenocytes or resident peritoneal cells was measured by a radioimmunoassay technique utilizing anti-PGE_2 antisera and [^{3}H] PGE_2 in a competitive binding technique. The adherent spleen cells were incubated with 5, 10, 50 or 100 µg/ml UDMH for two hrs, then washed and further incubated for 24, 48, 72 or 96 hrs. The peritoneal cells were incubated in the presence of 100 ng/ml lipopolysaccharide (for activation) and 0 (control), 5, 10, 25, 50, 75 or 100 µg/ml UDMH for 24 or 48 hrs. The supernate from all the cell cultures was assayed for PGE_2 levels.

2. Results

a. Adherent spleen cells: After the 24 hr incubation period there was a significant decrease in supernate PGE_2 levels at 10, 50 and 100 µg/ml. With longer incubation periods (48-96 hrs) there were increases in PGE_2 levels, particularly at 48 hrs (Table 5).

b. Resident peritoneal macrophages: There was a marked concentration-related decrease in PGE_2 levels after 24 and 48 hr incubation periods with UDMH. The data for 24 hrs is expressed in Figure 1 as percent of control (untreated) cells.

3. Discussion

The decrease in PGE_2 production by adherent splenocytes (24 hrs after UDMH exposure) and peritoneal cells could at least partly explain the immunoenhancement effects of UDMH. PGE_2 is known to suppress many immune functions, hence a decrease in normal PGE_2 levels allows for augmentation of the immune response. The enhancement of PGE_2 levels after 48-96 hrs of incubation may reflect a non-specific stimulation of the macrophages at longer culture periods. However, the time during the immune response at which PGE_2 exerts its effect is during the induction phase which occurs during the first 24 hrs, so this is the significant time period as far as the immune response is concerned.

E. Effects of UDMH on intracellular (spleen cell) cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP).

1. Background and methodology

Many agents which influence immunocyte function do so by altering intracellular cyclic nucleotide levels (cyclic adenosine monophosphate or cAMP, and cyclic guanosine monophosphate, or cGMP). For instance, PGE_2 is thought to inhibit mitogen-induced lymphocyte proliferation by inducing an increase in cellular cAMP. Conversely, mitogen-induced lymphocyte proliferation is associated with an increase in cellular cGMP.

Cyclic nucleotide levels are determined by performance of a radioimmunoassay on precipitated cellular protein. The type of assay is similar as described above for PGE_2. In these experiments, spleen cells were incubated with 0 (control), 5, 10^2, 25, 50, 75 or 100 µg/ml UDMH for 2 hrs, then washed and incubated alone or with the mitogens concanavalin A or lipopolysaccharide for 10 minutes, 1 hr, 4 hrs, or 24 hrs. Results were expressed as fm/µg cellular protein.

2. Results

Preliminary examination of the data from these experiments suggests that UDMH had no consistent or significant effect on cAMP or cGMP levels. However, values were quite variable between different experiments and samples were only run in duplicate for each experiment (due to large numbers of cells needed). We are currently looking at other ways of examining this data (such as percent of control) to see if any effects of UDMH may be masked by the experimental variability.

F. Effects of UDMH on autologous (syngeneic) mixed lymphocyte reaction (MLR)

1. Background and methodology

The autologous MLR has recently been described in normal humans, mice and guinea pigs. Purified T-cells will mount a proliferative response to purified B-cells (or other Ia antigen-bearing cells such as macrophages) from the same individual (autologous) or strain (syngeneic). Autoimmune strains of mice and humans with autoimmune diseases have a markedly suppressed autologous MLR. The inciting antigen is the Ia antigen expressed on the B-cell or macrophage surface.

After having tried several published methods for the syngeneic MLR, we now have one working in our laboratory. Spleen cells from Balb C mice are passed through a nylon wool column and the non-adherent cells constitute a T-cell enriched population which is used for the responder cells. To prepare stimulator cells, spleen cells (Balb C) are fractionated on a gradient of bovine serum albumin and the appropriate fraction (enriched for macrophages) is inactivated with mitomycin C. The two populations are cultured together for 4 or 5 days. Proliferative response is determined by adding tritiated thymidine to each culture and measuring its uptake into DNA.

2. Results

We have just begun evaluating UDMH in the syngeneic MLR. Preliminary results indicate that low concentrations of UDMH cause a slight enhancement of the SMLR and higher concentrations suppress the SMLR, when the UDMH is present during the entire culture period. Further experiments will involve pre-incubation of both responder and stimulator cell populations together, as well as each population separately. The SMLR of UDMH-treated mice will also be evaluated.

G. Effects of UDMH on interleukin production and activity.

Interleukin 1 (IL1) (also called lymphocyte activating factor) is produced by activated macrophages (which occurs upon exposure to mitogens). IL1 in turn induces interleukin 2 (IL2) (also called T-cell growth factor) production by T-lymphocytes, which spurs blastogenesis by mitogen-primed lymphocytes. Interleukin 1 production and activity can be assayed by stimulating monocytes with lipopolysaccharide, then harvesting the supernatant (containing IL1) and adding it to thymocyte cultures. Thymocyte proliferation is determined by measuring tritiated thymidine uptake in DNA. Interleukin 2 production and activity can be assayed by incubating purified T-cells with IL1 (activated monocyte supernatant) and concanavalin A (con A), then harvesting this supernatant and adding it to cultures of con A-primed T-cells and measuring tritiated thymidine uptake.

We are just beginning to develop these assays in our laboratory. Once they are functioning we will evaluate the effects of UDMH on both production of interleukins and their action on target cells.

III. Written Publications (cumulative list)

A. Suppression of mitogen-induced blastogenesis of feline lymphocytes by in vitro incubation with carcinogenic nitrosamides. Tarr, M.J. and Olsen, R.G. Immunopharmacology 2:191-199, 1980.

B. Differential effects of hydrazine compounds on B- and T-cell immune function. Tarr, M.J. and Olsen, R.G. AGARD Conference Proceedings No. 309, Toxic Hazards in Aviation, B3-1-7, 1981.

C. In vivo and in vitro effects of 1,1-dimethylhydrazine on selected immune functions. Tarr, M.J., Olsen, R.G. and Jacobs, D.L. Immunopharmacology 4:139-147, 1982.

D. Species variation in susceptibility to methylnitrosourea-induced immunosuppression. Tarr, M.J. and Olsen, R.G. In press, J. Env. Path. and Toxicol. 1984.

E. Comparison of in vitro and in vivo immunotoxicology assays. Tarr, M.J., Olsen, R.G. and Jacobs, D.L. Annals N.Y. Acad. Sci. 407:469-471, 1983.

IV. Professional Personnel Assoociated with Research Effort

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Debra L. Jacobs, B.S., M.S., Research Assistant

Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio 43210. Received M.S. degree from this department in March, 1982. Thesis title: Effects of 1,1-unsymmetrical dimethylhydrazine on the murine mixed lymphocyte reaction. Ms. Jacobs worked on this contract until June 24, 1983 as a research assistant.

Grace Sutter, B.S., Research Assistant

Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio 43210. Worked on this contract until August 12, 1983.

Brian Bowen, B.S., Graduate Research Associate

Mr. Bowen is working towards his Master of Science degree. He began working on this contract June 27, 1983.

Brenda McKown, B.S., Graduate Research Associate

Ms. McKown is working towards her Master of Science degree. She began working on this contract April 18, 1983.

V. Oral Presentations

A. Decreased Prostaglandin Release by Adherent Murine Splenocytes during Incubation with 1,1-Dimethylhydrazine. Tarr, M.J., Olsen, R.G. and Fertel, R. American Association for Cancer Research Annual Meeting, San Diego, CA, May 25-28, 1983.

B. Possible Explanations for the Immunoenhancement Effects of 1,1-Dimethylhydrazine. Tarr, M.J. and Olsen, R.G. Review of Air Force Sponsored Basic Research in Biomedical Sciences, University of California at Irvine, July 26-28, 1983.

Table 1. Effects of UDMH on expression of Thy 1.2 on murine spleen cells.

Concentration UDMH (µg/ml)	2 hr	4 hr	24 hr
0	43.3 ± 9.8	44.9 ± 12.4	67.1 ± 15.8
5	43.7 ± 8.9	43.1 ± 8.8	66.7 ± 13.7
10	45.9 ± 11.0	46.8 ± 12.3	58.9 ± 5.9
25	49.8 ± 11.6	47.9 ± 7.7	63.1 ± 9.6
50	46.8 ± 11.6	47.2 ± 13.8	67.3 ± 11.1
75	47.6 ± 8.2	49.9 ± 9.8	68.0 ± 10.7
100	48.4 ± 12.7	42.1 ± 8.3	62.2 ± 12.8
200	47.4 ± 8.4	45.7 ± 9.3	72.9 ± 15.8

Table 2. Effects of UDMH on Lyt 1.2 and Lyt 2.2 expression on murine spleen cells

Concentration UDMH (µg/ml)	1 hr		2 hr		4 hr	
	Lyt 1.2	Lyt 2.2	Lyt 1.2	Lyt 2.2	Lyt 1.2	Lyt 2.2
0 (Control)	36.4±10.9	25.4±10.9	24.1±6.9	25.4±8.6	37.9±12.9	26.0±8.8
5	39.6±9.3	28.5±8.4	29.1±2.0[a]	28.8±7.1	36.9±14.2	25.3±5.6
10	31.1±13.1	27.9±8.2	37.6±9.3[b]	31.3±6.9	39.8±13.7	24.2±7.3
25	36.7±12.9	30.6±14.8	40.9±9.1[b]	24.1±7.0	34.5±15.1	27.5±9.3
50	33.4±12.0	30.0±8.7	35.5±7.5[b]	24.5±4.4	38.8±17.1	31.2±7.4
75	37.2±12.1	31.8±8.4	35.4±4.3[b]	23.1±8.0	42.3±13.7	26.5±6.7
100	43.5±13.0	33.6±4.7	35.8±4.9[b]	23.4±6.4	41.3±13.7	28.9±9.9
200	38.9±13.7	30.6±7.8	34.7±6.7[b]	25.1±10.5	42.0±14.7	30.1±7.2

[a] $p = 0.011$ (student "t' test)

[b] $p < .001$

Table 3. Effects of UDMH on capping of concanavalin A by murine splenocytes

Concentration UDMH (µg/ml)	Percent Capping				
	45 min	2 hr	4 hr	24 hr	48 hr
0 (control)	56.0±6.5	68.5±7.5	58.3±3.0	35.0±3.7	33.5±4.7
5	64.3±4.3	60.0±5.7	63.3±5.1	34.3±10.8	34.0±10.3
10	67.3±6.1	61.5±9.3	61.3±6.3	36.0±7.1	31.3±14.7
25	61.5±7.0	63.5±5.1	61.3±2.6	27.0±4.0[a]	30.5±10.4
50	56.0±4.8	61.0±3.7	57.3±4.8	27.0±6.1	36.0±12.2
75	59.5±8.5	61.0±5.0	61.5±7.9	23.8±5.0[a]	33.5±10.5
100	55.5±7.5	62.5±2.4	54.8±14.3	18.3±7.0[a]	39.3±13.8
200	56.0±2.9	56.0±8.3	48.0±9.9	16.7±10.0[a]	40.7±14.9

[a] $p < .025$ (student "t" test)

Table 4. Effects of 2 hr preincubation with UDMH on percent of adherent splenocytes expressing Ia antigen

Concentration UDMH (ug/ml)	Percent of cells expressing Ia antigen			
	24 hr. inc.	48 hr. inc.	72 hr. inc.	96 hr. inc.
0 (control)	71.9 ± 5.4	71.7 ± 4.4	69.4 ± 7.2	71.6 ± 5.3
5	77.8 ± 7.0[a]	76.2 ± 4.6	80.3 ± 4.8[b]	67.1 ± 6.0
10	69.7 ± 4.2	82.8 ± 2.5[c]	79.8 ± 7.4[d]	71.4 ± 8.6
50	71.2 ± 5.3	75.9 ± 4.6	74.2 ± 6.6	74.5 ± 8.9
100	80.0 ± 6.0[b]	79.5 ± 4.8[d]	77.0 ± 4.6	77.6 ± 7.0

[a] $p < .05$

[b] $p < .025$

[c] $p < .005$

[d] $p < .01$

Table 5. Effects of 2 hr preincubation with UDMH on PGE_2 production by adherent splenocytes

Concentration UDMH (µg/ml)	PGE_2 (pg/10^6 spleen cells)			
	24 hr. inc.	48 hr. inc.	72 hr. inc.	96 hr. inc.
0 (control)	588 ± 110	515 ± 84	144 ± 44	218 ± 21
5	460 ± 99	670 ± 84[b]	196 ± 49	235 ± 40
10	267 ± 52[a]	706 ± 80[c]	236 ± 49[d]	287 ± 50[d]
50	228 ± 42[a]	734 ± 91[c]	463 ± 70[a]	251 ± 51
100	297 ± 66[a]	242 ± 24[a]	204 ± 51	238 ± 82

[a] $p < .001$ (student "t" test)

[b] $p < .025$

[c] $p < .005$

[d] $p < .05$

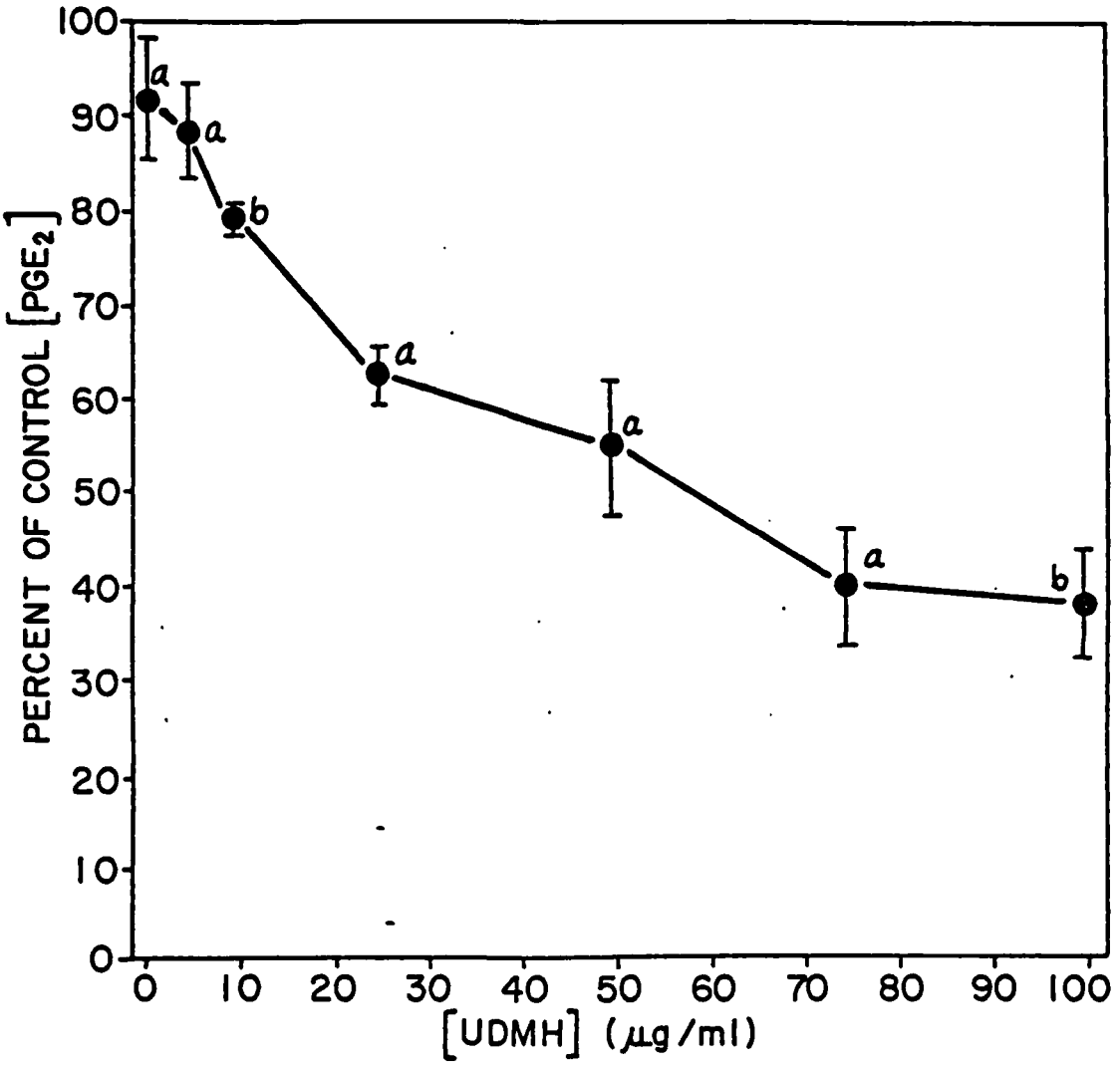

Figure 1. Effects of UDMH on PGE_2 Production by Resident Peritoneal Macrophages (RPM).

RPM were incubated for 24 hours with 100 ng lipopolysaccharide and UDMH. Supernatant was assayed for PGE_2 levels. Results are expressed as percent of control (untreated) RPM response.

[a] $p < 0.025$
[b] $p < 0.001$

Significance

Research during the FY 1982-83focused on delineating the mechanism of UDMH interference with immunoregulatory functions. The rationale of our experimental approach was to determine if UDMH was cytotoxic to suppressor cells, therefore altering the suppression/helper ratio. Our evidence indicated that spleen cells exhibiting suppressor cell markers were not altered. In conjunction with this research we attempted to ascertain whether the signals (receptors) on suppressor cells (as well as B-cell and macrophage) (Ia) were affected by UDMH exposure. The number of cells expressing Ia markers were slightly enhanced by UDMH. At this time no explanation is available as to the cell type expressing the Ia marked in our experimental system.

Recent data strongly suggest that UDMH inhibits PGE_2 synthesis. The prostaglandin is known to play a key role in immunoregulation. This finding may permit us to explore the biochemical events that lead to hydrazine-induced suppressor cell dysfunction. Moreover, this avenue of research may lead to methods of pharmacological reversal of hydrazine immunotoxicity.

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