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Induction of suppressor macrophages in mice by Fusarenon-X

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Summary. Intraperitoneal injection of Fusarenon-X into BALB/c mice, a mycotoxin produced by *Fusarium nivale* Fn 2B, depressed polyclonal antibody formation of splenic lymphocytes in response to pokeweed mitogen (PWM). This inhibitory activity was found to reside in the surface immunoglobulin-negative spleen cell fraction of Fusarenon-X-treated mice, sIg^-(FX), which comprised mainly T lymphocytes and smaller number of non-lymphocytic cellular elements. However, reconstitution experiments for *in vitro* antibody formation provided evidence that T lymphocytes from Fusarenon-X-treated mice, T(FX), which were separated from non-lymphocytic cells by use of carbonyl iron/magnet, were as effective as T lymphocytes from normal mice, T(N), in supporting antibody formation. Furthermore, addition of non-lymphocytic cells, NL(FX), or adherent cells, AD(FX), prepared from spleen cells of Fusarenon-X-treated mice to normal spleen cells strongly inhibited *in vitro* antibody formation against PWM or a bacterial lipopolysaccharide (LPS).

These results strongly indicated that Fusarenon-X induced non-lymphocytic suppressor cells in the spleen of the treated mice which had features in common with activated macrophages.

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INTRODUCTION

Fusarenon-X is one of the trichothecene mycotoxins isolated from *Fusarium nivale* Fn 2B and characterized as 3α, 7α, 15-trihydroxy-4β-acetoxy-8-oxo-12, 13-epoxy-trichotec-9-ene (Ueno, Ueno, Tatsuno, Ohokubo & Tsunoda, 1969). T_2-toxin and diacetoxyscirpenol, other trichothecene mycotoxins, have been reported earlier to have suppressive effect on anti-SRBC antibody formation and allograft rejection in mice (Rosenstein, Lafarge-Frayssinet, Lespinats, Loisillier, Lafont & Frayssinet, 1979).

Previous reports from our laboratories have also demonstrated that the treatment of mice *in vivo* or spleen cells *in vitro* with Fusarenon-X depressed the proliferative response of splenic lymphocytes to T-cell mitogen and to lesser extent to B-cell mitogen. The treatment of mice with Fusarenon-X also inhibited anti-DNP IgE and IgG1 antibody formation. Furthermore, we demonstrated that mitogen-induced *in vitro* antibody formation was depressed in the spleen cells of Fusarenon-X-treated mice and showed that the inhibitory activity was attributable to the sIg^- spleen cell fraction (Masuda, Takemoto, Tatsuno & Obara, 1982).

The present study further investigated the immunosuppressive effect of Fusarenon-X and shows that the inhibitory cells present in the spleen of Fusarenon-X-treated mice were not lymphocytes but most likely activated macrophages and/or other cellular elements such as granulocytes which were also increased in the spleen by the treatment.

MATERIALS AND METHODS

Animals

Six-week-old male mice of BALB/c inbred line were obtained from Charles River Co., Ltd, Japan and used for the experiments at 7–9 weeks of age.

Materials

Fusarenon-X isolated and purified as described previously (Tatsuno, 1968; Fujimoto, Morita & Tatsuno, 1972) was dissolved in saline before use. Pokeweed mitogen (PWM) and *E. coli* lipopolysaccharide (LPS) were purchased from Grand Island Biological Company, New York and Sigma Biochemicals, Mc., U.S.A., respectively. RPMI 1640 medium was obtained from Nissui Co., Ltd, Japan. Rabbit anti-mouse Ig antibody and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse Ig antibody (GAMIG) and rabbit anti-mouse thymocyte antibody (RAMT) were purchased from Cappel Laboratories Inc. Penn., U.S.A.

Treatment with Fusarenon-X

Animals were treated i.p. with 50 μg Fusarenon-X daily for 7 days unless otherwise indicated.

Preparation of spleen cells

Mouse spleens were aseptically removed and gently dissected by using forceps in RPMI 1640 medium. The spleen cells were filtered through nylon gauze, treated with tris-buffered NH_4Cl to lyse the erythrocytes and then washed three times and resuspended in RPMI 1640 medium containing 10% foetal calf serum (FCS), 25 mM N-2-hydroxyethylpiperadine-N′-2-ethanesulphonic acid (HEPES) and 50 μM 2-mercaptoethanol (2-ME). The number of viable cells was assessed by trypan blue dye exclusion.

Preparation of sIg negative (sIg^-) and positive (sIg^+) cells

sIg^- and sIg^+ spleen cells were prepared according to the method of Wysocki & Sato (1978) as described previously (Masuda *et al.*, 1982). Spleen cells (3×10^7) prepared as above were incubated in polystyrene culture dishes coated with rabbit anti-mouse Ig antibody for 70 min at 4°. The non-adherent cells which did not attach to the substratum and the adherent cells which were detached by flushing off with a pipette were designated as sIg^- and sIg^+, respectively.

T lymphocytes from Fusarenon-X-treated mice

Spleen cells from Fusarenon-X-treated mice were resuspended in RPMI 1640 medium containing 10% FCS to give 5×10^7 cells/ml and incubated in the presence of 25 mg/ml carbonyl iron (Wako Junyaku Co., Ltd, Japan) for 1 hr at 37°. Non-phagocytic cells floating in the medium were separated from phagocytic cells attached to the substratum by a magnet placed under the culture dishes. The non-phagocytic cells collected were placed again on the magnet and this procedure was repeated three times for obtaining a lymphocyte-rich cell population. The non-phagocytic cells thus obtained were further separated by use of an antibody-coated culture dish and the cells which did not bind to this were designated as T(FX).

Non-lymphocytic cells: NL (FX)

Spleen cells prepared from Fusarenon-X-treated mice were resuspended in RPMI 1640 medium to give 5×10^7 cells/ml. Lymphocytes present in the spleen cell preparation were lysed by incubation with rabbit anti-mouse thymocyte antibody (RAMT) for 30 min at 4°, followed by incubation for 1 hr at 37° in the presence of guinea-pig complement. By this procedure, not only T but also B lymphocytes were removed from the spleen cell preparation. The remaining viable cells were washed three times with RPMI 1640 before use. The recovery of viable non-lymphocytic cells was 3·6% and the contamination with lymphocytes was 2·4%.

Adherent cells: AD(FX)

Spleen cells from Fusarenon-X-treated mice (1×10^8 cells in 2 ml of RPMI 1640 medium containing 50% FCS) were plated into a culture dish (Nunc Inter Med, Denmark, 6 cm in diameter) and incubated for 2 hr at 37° under 5% CO_2 atmosphere. Non-adherent cells were removed by washing the plate three times with RPMI 1640 medium containing 10% FCS. The cells strongly adherent to the substratum were then collected from the plate by incubating with PBS containing 5% FCS and 0·2% ethylenediamine tetraacetic acid (EDTA-Na_2) for 15 min at 4°. The adherent cells recovered were washed three times with RPMI 1640 medium before use.

Antibody-forming cells

Antibody-forming cells with positive cytoplasmic staining by FITC-conjugated rabbit anti-mouse Ig antibody were determined as described previously (Masuda *et al.*, 1982). Briefly, spleen cells or sIg^+ and sIg^- cells (5×10^6 cells in a typical culture) suspended in RPMI 1640 medium with 10% FCS, 25 mM HEPES

and 50 μM 2-mercaptoethanol were stimulated by PWM (1:200 dilution) or LPS (100 μg/ml) and incubated at 37° under 5% CO_2 atmosphere. Smears were made and stained with FITC-conjugated goat anti-mouse Ig antibody and the number of cells with positive cytoplasmic staining was counted. Antibody-forming cells were enumerated by multiplying the number of total viable cells contained in each spleen cell culture by percentage of cytoplasmic Ig^+ cells counted on more than 500 spleen cells. Culture of spleen cells with PWM or LPS gave about ten-fold stimulation in generation of intracytoplasmic immunoglobulin positive cells compared with control culture without mitogen.

RESULTS

The number of antibody-forming cells was evaluated by in vitro stimulation of spleen cells with PWM after repeated i.p. injection of 50 μg Fusarenon-X into BALB/c mice. As shown in Fig. 1, the control culture gave over 2×10^5 antibody-forming cells with positive immunoglobulin staining in cytoplasm. However, the number of antibody-forming cells decreased to almost half the control level after a single injection of Fusarenon-X. The antibody response was suppressed to a greater extent when the cells were prepared from mice which had received repeated administrations of Fusarenon-X. Surface immunoglobulin positive and negative spleen cells from normal and Fusarenon-X-treated mice were separated and examined for generation of antibody-forming cells after their reconstitution. It was clearly shown that suppressor activity was present in the surface immunoglobulin-negative spleen cell fraction of Fusarenon-X-treated mice, sIg^-(FX) (Table 1), as reported previously (Masuda *et al.*, 1982).

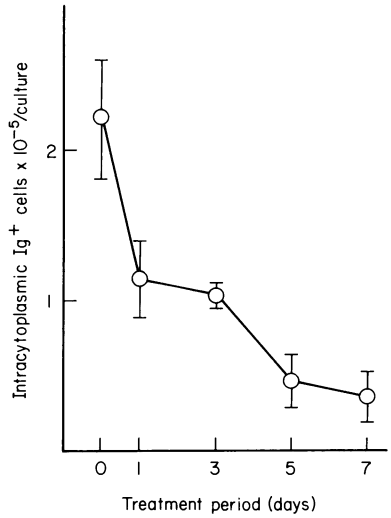

Figure 1. Effect of administration of Fusarenon-X on induction of antibody-forming cells by PWM. Five groups of BALB/c mice, each comprising three animals, were treated i.p. with 50 μg Fusarenon-X daily for the indicated number of days. Spleens were removed one day after the final injection and spleen cells (5×10^6) were incubated with PWM for 96 hr. Cytoplasmic Ig-containing cells stained with FITC-GAMIG were determined. Each point represents mean determined on three separate spleens; vertical bar indicates SD of the mean.

Table 1. Suppressor activity of sIg^- cell fraction from Fusarenon-X-treated mice in the induction of antibody-forming cells by PWM

Composition of the cell fraction*					Intracytoplasmic Ig^+ cells‡ ($\times 10^{-4}$/culture)
sIg^+(N)	($2{\cdot}5 \times 10^6$)†	+	sIg^-(N)	($2{\cdot}5 \times 10^6$)	$10{\cdot}5 \pm 1{\cdot}2$
		+	sIg^-(FX)	($2{\cdot}5 \times 10^6$)	$0{\cdot}7 \pm 0{\cdot}2$
S (N)	($5{\cdot}0 \times 10^6$)				$7{\cdot}2 \pm 1{\cdot}1$
sIg^+(FX)	($2{\cdot}5 \times 10^6$)	+	sIg^-(N)	($2{\cdot}5 \times 10^6$)	$6{\cdot}4 \pm 0{\cdot}3$
		+	sIg^-(FX)	($2{\cdot}5 \times 10^6$)	$0{\cdot}7 \pm 0{\cdot}0$
S(FX)	($5{\cdot}0 \times 10^6$)				$1{\cdot}5 \pm 0{\cdot}5$

* sIg^+(FX), sIg^-(FX) and S(FX) represent surface Ig positive, surface Ig negative or unseparated spleen cells prepared from mice treated i.p. with 50 μg Fusarenon X daily for 7 days. sIg^+(N), sIg^-(N) and S(N) are surface Ig positive, surface Ig negative or unseparated spleen cells taken from normal mice.

† Cell number.

‡ Mean ± SD of duplicate cultures.

Since the sIg^- cell fraction consisted mainly of T lymphocytes, a possible alteration of T-cell function by the treatment was examined. As shown in Table 2, T cells prepared from spleen cells of Fusarenon-X-treated mice, T(FX), by removing phagocytic cells with the use of carbonyl iron/magnet and further separating B cells by Ig-coated dishes, were as effective as T cells from normal mice in supporting antibody formation. These results indicated that no functional changes such as abrogation of helper T-cell activity or induction of suppressor T-cell activity were introduced by the treatment in the splenic lymphocytes. They strongly suggested that the suppressor activity was very likely attributable to non-lymphocytic cellular elements present in sIg^-(FX) cell fraction such as macrophages.

Non-lymphocytic cells, NL(FX), were therefore prepared from spleen cells of Fusarenon-X-treated mice by treatment with rabbit anti-mouse thymocyte antibody and complement. The resulting NL(FX) consisted mainly of macrophages and granulocytes such as neutrophils. Table 3 shows that addition of

Table 2. Lack of suppressor activity of T-cell fraction separated from sIg^- (FX) cell fraction in the induction of antibody-forming cells by PWM

Composition of the cell fraction*					Intracytoplasmic Ig^+ cells‡ ($\times 10^{-4}$/culture)
sIg^+(N)	$(2{\cdot}5\times10^6)$†	+	T (N)	$(2{\cdot}5\times10^6)$	$18{\cdot}4\pm1{\cdot}9$
		+	T (FX)	$(2{\cdot}5\times10^6)$	$19{\cdot}6\pm0{\cdot}4$
		+	sIg^-(FX)	$(2{\cdot}5\times10^6)$	$3{\cdot}4\pm0{\cdot}1$
S (N)	$(5{\cdot}0\times10^6)$				$15{\cdot}8\pm2{\cdot}4$
S (FX)	$(5{\cdot}0\times10^6)$				$0{\cdot}7\pm0{\cdot}1$
sIg^+(FX)	$(2{\cdot}5\times10^6)$	+	T (N)	$(2{\cdot}5\times10^6)$	$9{\cdot}7\pm0{\cdot}6$
		+	T (FX)	$(2{\cdot}5\times10^6)$	$9{\cdot}1\pm0{\cdot}8$
		+	sIg^-(FX)	$(2{\cdot}5\times10^6)$	$1{\cdot}7\pm1{\cdot}4$

* sIg^+(FX), sIg^-(FX), S(FX), sIg^+(N), sIg^-(N) and S(N) are the cells similarly prepared as described in Table 1. T(FX) and T(N) are non-phagocytic surface Ig negative spleen cells from mice treated daily with 50 μg Fusarenon X for 7 days or from normal mice, respectively.

† Cell number.

‡ Mean ± SD of duplicate cultures.

Table 3. Suppressor activity of non-lymphocytic cell fraction from Fusarenon-X-treated mice in the induction of antibody-forming cells by PWM or LPS

Mitogen	Composition of the cell fraction*							Intracytoplasmic Ig^+ cells‡ ($\times 10^{-4}$/culture)
PWM	sIg^+(N)	$(2{\cdot}5\times10^6)$†	+	sIg^-(N)	$(1{\cdot}25\times10^6)$			$8{\cdot}1\pm0{\cdot}3$
			+	sIg^-(N)	$(1{\cdot}25\times10^6)$	+ NL (FX)	$(1{\cdot}25\times10^6)$	$1{\cdot}8\pm0{\cdot}2$
	sIg^+(FX)	$(2{\cdot}5\times10^6)$	+	sIg^-(N)	$(1{\cdot}25\times10^6)$			$5{\cdot}3\pm0{\cdot}2$
			+	sIg^-(N)	$(1{\cdot}25\times10^6)$	+ NL(FX)	$(1{\cdot}25\times10^6)$	$1{\cdot}1\pm0{\cdot}1$
LPS	sIg^+(N)	$(5{\cdot}0\times10^6)$						$43{\cdot}9\pm3{\cdot}4$
			+	NL (FX)	$(1{\cdot}25\times10^6)$			$1{\cdot}5\pm0{\cdot}2$
	sIg^+(FX)	$(5{\cdot}0\times10^6)$						$23{\cdot}6\pm1{\cdot}8$
			+	NL (FX)	$(1{\cdot}25\times10^6)$			$1{\cdot}1\pm0{\cdot}2$
	S (N)	$(5{\cdot}0\times10^6)$						$30{\cdot}0\pm2{\cdot}2$
	S (FX)	$(5{\cdot}0\times10^6)$						$2{\cdot}6\pm0{\cdot}2$

* sIg^+(FX), S(FX), sIg^+(N), sIg^-(N) and S(N) were prepared as described in Table 1. NL(FX), non-lymphocytic spleen cells from Fusarenon-X-treated mice, were prepared as described in Materials and Methods.

† Cell number.

‡ Mean ± SD of duplicate cultures.

NL(FX) strongly inhibited the generation of antibody-forming cells in response to either PWM or LPS. Furthermore, when 5×10^6 spleen cells from normal mice were admixed with various numbers of NL(FX) and stimulated with PWM or LPS, generation of antibody-forming cells was inhibited to an extent depending on the number of NL(FX) added to the culture (Fig. 2). One tenth of the number of normal spleen cells was sufficient for NL(FX) to suppress antibody response to both PWM and LPS by more than 50%. Similarly, addition of adherent cells prepared from Fusarenon-X-treated mice to normal spleen cells also inhibited antibody formation in response to PWM or LPS (Table 4).

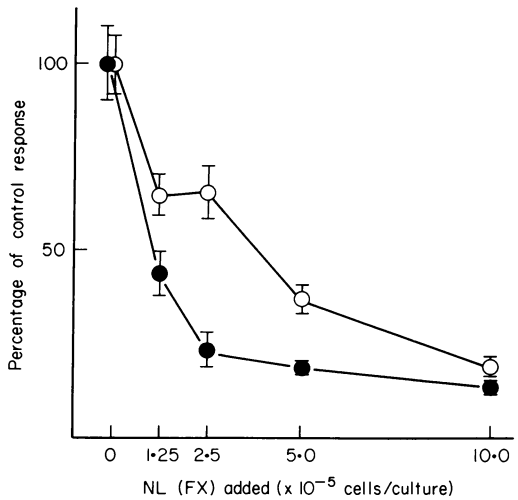

Figure 2. Effect of non-lymphocytic cells from Fusarenon-X-treated mice on induction of antibody-forming cells by PWM or LPS. Non-lymphocytic cells, NL(FX), were prepared from spleen cells of Fusarenon-X-treated mice by treatment with RAMT and complement. Spleen cells (5×10^6) from untreated mice were incubated with PWM (●) or LPS (○) for 96 hr in the presence of various numbers of NL(FX). The response was shown as percentage of the control responses without addition of non-lymphocytic cells, which were $(1{\cdot}50 \pm 0{\cdot}14) \times 10^5$ positive cells/culture for PWM stimulation and $(1{\cdot}56 \pm 0{\cdot}12) \times 10^5$ positive cells/culture for LPS stimulation. Each point and vertical bar indicate mean ± SD of duplicate cultures.

In order to determine whether the suppressor cells present in NL(FX) are required for suppression at the time of mitogen-stimulation or whether they are also effective at later stage, NL(FX) was added to normal spleen cells at various times after initiation of culture. As shown in Table 5, the suppression was most evidently observed when NL(FX) was present during the initial 24 hr after PWM stimulation. Addition of NL(FX) after 48 hr or later was ineffective in suppressing the antibody formation against PWM. Similar results were obtained with LPS although it was less effective when added 24 hr after mitogen stimulation.

DISCUSSION

Previous studies from our laboratories have shown that Fusarenon-X depressed the lymphoproliferative response of spleen cells to T- and B-cell mitogens and suppressed anti-DNP IgE and IgG1 antibody production *in vivo* (Masuda *et al.*, 1982). It was noted that non-lymphocytic cellular elements were prominently increased in the spleen of Fusarenon-X-treated mice.

By utilizing a reconstitution system for *in vitro*

Table 4. Suppressor activity of adherent cells from Fusarenon-X-treated mice in the induction of antibody-forming cells by PWM or LPS

Cell source*		Intracytoplasmic Ig^+ cells ($\times 10^{-4}$/culture)‡ induced by PWM	LPS
S (N)	$(5{\cdot}0 \times 10^6)$†	$14{\cdot}2 \pm 1{\cdot}7$	$15{\cdot}1 \pm 1{\cdot}2$
S (N)	$(5{\cdot}0 \times 10^6)$ + AD (FX)$(1{\cdot}0 \times 10^6)$	$1{\cdot}2 \pm 0{\cdot}5$	$4{\cdot}5 \pm 0{\cdot}3$
S (FX)	$(5{\cdot}0 \times 10^6)$	$1{\cdot}0 \pm 0{\cdot}3$	$4{\cdot}4 \pm 0{\cdot}6$

* S(N), unfractionated spleen cells from normal mice. AD(FX), adherent cells from spleen of Fusarenon-X-treated mice were prepared as described in Materials and Methods.

† Cell number.

‡ Mean ± SD of duplicate cultures.

Table 5. Effect of time of addition of non-lymphocytic cells from Fusarenon-X-treated mice on induction of antibody-forming cells by PWM or LPS

Mitogen	Incubation time (hr)	Cell source*	Addition of NL(FX)†	(time)	Intracytoplasmic Ig^+ cells‡ ($\times 10^{-4}$/culture)	(% of control)
PWM	96	S(N)	—		7·3±0·3	(100)
			+	(0 hr)	2·2±0·1	(30)
			+	(24 hr)	3·6±0·5	(49)
			+	(48 hr)	8·8±0·4	(121)
			+	(72 hr)	7·4±0·4	(101)
		S(FX)	—		0·1±0·1	(1)
LPS	72	S(N)	—		12·7±0·9	(100)
			+	(0 hr)	3·2±0·4	(25)
			+	(24 hr)	10·4±0·4	(82)
			+	(48 hr)	11·0±1·8	(87)
		S(FX)	—		2·7±0·5	(21)
	96	S(N)	—		15·1±2·3	(100)
			+	(0 hr)	5·1±1·0	(34)
			+	(24 hr)	12·0±0·6	(79)
			+	(48 hr)	13·2±1·9	(87)
			+	(72 hr)	18·3±1·3	(121)
		S(FX)	—		1·8±0·5	(12)

* S(N) and S(FX) were prepared as described in Table 1 (5×10^6 cells).

† NL(FX), non-lymphocytic cells from Fusarenon-X-treated mice (1×10^6 cells), were added to S(N) at various times after initiation of culture as indicated.

‡ Mean ± SD of duplicate cultures.

antibody formation, the present study provided evidence that the immunosuppressive activities resided in the sIg^- spleen cell fraction of Fusarenon-X-treated mice which was enriched with T lymphocytes. However, functional activities of T lymphocytes in terms of suppressor or helper activity were shown to be unaffected by the treatment; T cells prepared from spleen cells of Fusarenon-X-treated mice supported *in vitro* antibody formation by PWM as well as T cells from normal mice when they were reconstructed with sIg positive B lymphocytes of normal or Fusarenon-X-treated mice. These results strongly indicated that T- and B-cell functions were not altered by *in vivo* treatment with Fusarenon-X and suggested further that non-lymphocytic cells, which increased in the spleen after Fusarenon-X treatment, were the cells responsible for the observed inhibition.

In fact, addition of non-lymphocytic cells prepared from spleen cells of Fusarenon-X-treated mice to the reconstituted culture system or normal spleen cells markedly inhibited the generation of antibody-forming cells. Therefore, depression of the *in vitro* antibody response found in the spleen cells of Fusarenon-X-treated mice was accounted for by the increase of non-lymphocytic cells.

The increase of similar cellular elements in the spleen has been reported earlier on the animals bearing virus-induced tumours (Kirchner, Chused, Herberman, Holden & Lavrin, 1974a; Glaser, Kirchner & Herberman, 1975; Veit & Feldman, 1976b) or methylcholanthrene-induced tumours (Kirchner, Herberman, Glaser and Lavrin, 1974b) and in animals exposed to infectious agents (Scott, 1972; Kirchner, Holden & Herberman, 1975a; Bixler & Booss, 1981). In addition to suppressing the lymphoproliferative response, these cells were found to inhibit the mixed lymphocyte reaction (Veit & Feldman, 1976a; 1976 b) and tumour cell growth *in vitro* (Kirchner *et al.*, 1975a; Oeiiler, Campbell & Herberman, 1977) and to exhibit tumour cell cytotoxicity (Krichner *et al.*,1974a; Veit & Feldman, 1976; Oeiiler *et al.*, 1977; Glaser, Kirchner, Holden & Herberman, 1976). The generation of cytotoxic killer T cells (Glaser, Kirchner, Holden & Herberman, 1976) and the *in vitro* antibody response (Bixler & Booss, 1981) were also inhibited. The cells responsible for these inhibitory activities were claimed

to be macrophages, based on the facts that they were resistant to X-irradiation and to treatment with anti-theta or anti-thymocyte antibody and complement, but were removed by treatment with iron filings and magnet or passage through a nylon column.

The inhibitory cells present in NL(FX) appeared to be also macrophages since their inhibitory activity was depleted from spleen cells by iron filings and magnet but was resistant to the treatment with anti-thymocyte antibody and complement. Furthermore, addition of adherent cells from Fusarenon-X-treated mice to normal spleen cells inhibited antibody formation stimulated with mitogens.

NL(FX) was required to be present within 24 hr after mitogen stimulation for achievement of suppression of antibody formation. Interestingly, similar results were reported earlier on the activated macrophages which were found to be increased in the spleens of mice bearing Moloney sarcoma virus (MSV)-induced tumours. These macrophages were shown to inhibit the proliferative response of splenic lymphocytes to a T-cell mitogen, phytohaemagglutinin (PHA), only when they were added to normal spleen cells within 24 hr after mitogen stimulation (Kirchner, Mushmore, Chused, Holden & Herberman, 1975b). It seems likely that the suppression of the *in vitro* immune response observed in the spleens of Fusarenon-X-treated mice is also due to the increase of suppressor macrophages similar to the activated macrophages previously reported by others as described above.

However, it is also possible that granulocytes may play a role in the depression observed in the current study, because granulocytes were reported to release hydrogen peroxide which was suppressive to immune responses *in vitro* (Nathan, Brukner, Silverstein & Cohn, 1979a) and were also found to increase in the spleen of Fusarenon-X-treated mice. It is, however, more likely that the inhibitory activity of NL(FX) is mainly associated with macrophages on account of their adherent nature. It remains to be further clarified whether inhibition of the lymphoproliferative response and of *in vitro* antibody formation by Fusarenon-X results from direct interaction of the suppressor macrophages with lymphocytes or is mediated by liberation of inhibitory factor(s) such as prostaglandins and/or hydrogen peroxide (Grimm, Seitz, Kirchner & Gemsa, 1978, Nathan, Silverstein, Brukner & Cohn, 1979b; Metzger, Hoffeld & Oppenheim, 1980).

Our previous and present studies showed that administration of a mycotoxin, a small molecular weight compound produced by *Fusarium nivale* Fn 2B, caused immunological deficiency which was comparable to that shown in the animals bearing tumours or exposed to infectious agents. The deficiency could most likely be explained by the induction of suppressor macrophages in the spleen.

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