INDUCTION OF T CELLS PRODUCING MACROPHAGE MIGRATION INHIBITION FACTOR BY MINOR HISTOCOMPATIBILITY ANTIGENS

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Antigens of the principal histocompatibility complex, which constitute the chief barrier for transplantation, are controlled by genes located in mice on the 17th chromosome and are concentrated within the H-2 complex. Weaker minor transplantation H-antigens are controlled by many loci scattered throughout the mouse genome [1]. Differences with respect to minor H-antigens (non H-2) lead to rejection of the graft which takes place more slowly than in the presence of differences for H-2 antigens, and varying from 28 to 400 days. Antibodies causing immunologic enhancement or antibodies incapable of binding complement are present more frequently in antisera against minor H antigens, whereas strong hemagglutinating and cytotoxic antibodies are difficult to find [4].

It was shown previously that non-H antigens can induce a response of T killer cells and T helper cells [9], effectors of delayed-type hypersensitivity (DTH) [13], effectors of the local "graft versus host reaction" [14], and also production of macrophage migration inhibition factor (MIF) and of interferon in a mixed lymphocyte culture *in vitro* [15], although the question of induction of MIF producers by non H-2 antigens *in vivo* has not previously been studied.

The aim of this investigation was to study the ability of T cells producing MIF to respond to minor histocompatibility antigens during intravenous immunization *in vivo*.

EXPERIMENTAL METHOD

The genetic characteristics of the inbred lines of mice used in the work are given in Table 1. The selected pairs of lines of mice differed for the whole of H-2 and for minor, non H-2, H antigens (lines B6 and D2); for H-2 only (B10 and D2); for non H-2 antigens and identical with respect to the Mls locus (B6 and B10, D2 and BALB/c), and also for non H-2 antigens, and with differences relative to the Mls locus (BALB/c and DBA/2). The animals were immunized by a single intravenous injection of an irradiated (15 Gy) suspension (90×10^6 cells in 0.8 ml of Hanks' solution per mouse) of normal spleen cells from mice differing in H-2 and (or) non H-2 antigens from the immunized line, by a method developed by the writers [5]. On the 1st, 6th, and 13th days suspension of spleen cells were obtained from immune (in the control, from normal) animals and mixed in the ratio of 10:1 with spleen cells of the immunizing line (in the control, with syngeneic cells). The mixtures of cells were incubated for 18 h as described previously [8] and the culture fluids (CF) were separated from cells by centrifugation (800 g, 20 min) and studies in a micromodification of the macrophage migration inhibition test (MMIT) [2]. Activity of MIF in the CF studies was defined as the MMI index (MMII) by the equation:

$$\text{MMII} = \left(1 - \frac{\text{mean area of migration in experiment}}{\text{mean area of migration in control}}\right) \times 100\%.$$

The T-cell nature of MIF production on intravenous immunization with irradiated spleen cells *in vivo* was demonstrated by the writers previously [5, 8].

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TABLE 1. Brief Genetic Characteristics of Pairs of Mouse Lines Used in the Work for Immunization

Lines of mice	Abbreviation of lines	H-2 haplotypes	Genetic basis of line	Allele of Mls locus
C57BL/6	B6	b	B6	b
B10D2	D2	d	B10	b
C57BL/10Sn	B10	b	B10	b
B10.D2	D2	d	B10	b
C57BL/6	B6	b	B6	b
C57BL/10Sn	B10	b	B10	b
B10.D2	D2	d	B10	b
BALB/c	—	d	BALB/c	b
BALB/c	—	d	BALB/c	b
DBA/2	—	d	DBA/2	a

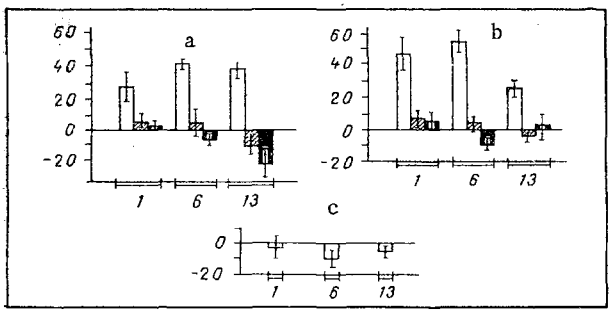

Fig. 1. Response of T cells producing MIF to products of H-2 (a), and H-2 and non H-2 antigens (b) and absence of response to syngeneic cells (c) after intravenous B10 anti-D2 (a), B6 anti-D2 (b), and B6 anti-B6 (c) immunization. Unshaded columns — response of immune cells to cells of immunizing line; obliquely shaded columns — response of immune cells to syngeneic cells, black columns — response of normal cells of recipient line to corresponding antigen: a, b (for D2), c) for B6. Vertical axis MMII (in %, M ± m); horizontal axis — days after immunization.

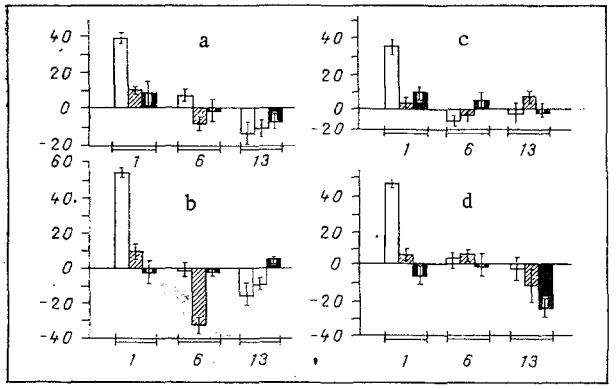

Fig. 2. Response of T cells producing MIF to non H-2 antigens identical for Mls locus at different times after intravenous immunization. a) BALB/c anti-D2; b) D2 anti-BALB/c; c) B6 anti-B10; d) B10 anti-B6. Black columns denote response of normal cells of recipient's line to corresponding antigen: a) for D2; b) for BALB/c; c) for B10; d) for B6. Remainder of legend as to Fig. 1.

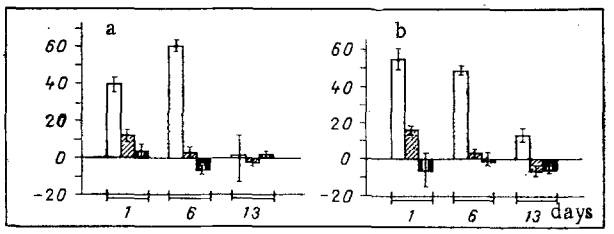

Fig. 3. Response of T cells producing MIF to non H-2 antigens differing for Mls locus. Unshaded columns — response of immune cells to cells of immunizing line: a) BALB/c anti-DBA/2 to DBA/2, b) DBA/2 anti-BALB/c to BALB/c; obliquely shaded columns — response of immune cells to syngeneic cells: a) BALB/c anti-DBA/2 to BALB/c. b) DBA/2 anti-BALB/c to DBA/2; black columns — response of normal cells of recipient's line to corresponding antigen; a) BALB/c to DBA/2, b) DBA/2 to BALB/c. Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

On intravenous immunization of a donor, differing from the recipient line as regards both H-2 and non H-2 antigens, with irradiated allogeneic spleen cells, activity of MIF producers could be detected on the 1st, 6th, and 13th days after immunization (Fig. 1b). Differences with respect to antigens of the H-2 complex, when the animals were identical for non H-2 antigens, also were sufficient to induce MIF producers at these same times (Fig. 1a), although the intensity of the response of these cells on the 1st and 6th days after immunization was somewhat lower than when differences were present for H-2 and non H-2 antigens simultaneously. Immunization with syngeneic cells did not induce the formation of MIF producers at all times of the investigation (Fig. 1c). When the donor and recipient lines differed with respect to non H-2 antigens but were identical with respect to H-2 and the Mls locus, MIF producers could be induced only on the 1st day, but not on the 6th or 13th days after immunization (Fig. 2). This feature of the kinetics of the response of MIF producers was demonstrated by reciprocal immunization of donor—recipient pairs with different H-2 haplotypes: B6 and B10 — $H2^b$; D2 and BALB/c — $H\text{-}2^d$ (see Table 1), indicating the universal nature of the phenomenon discovered.

Among non-H-2 antigens a special place is occupied by products of the Mls locus, which effectively stimulate T cell proliferation in mixed lymphocyte culture (MLC), but which induce the formation of cytotoxic T lymphocytes poorly [1]. As Fig. 3 shows, during reciprocal immunization of mice of lines BALB/c and DBA/2, differing for both non H-2 antigens and the Mls locus, a strong response of MIF producers was found on the 1st and 6th days, but not on the 13th day after immunization. In the presence of differences for the Mls locus in addition to other non H-2 antigens, the kinetics of formation of MIF producers thus differed from that when differences were present for non H-2 antigens except Mls products, or for H-2 antigens (Figs. 1 and 2). In all cases when a response of MIF producers was discovered, it developed only on repeated contact of the donor with the antigens; MIF production under these circumstances in the presence of syngeneic cells could not be found after any of the immunizations studied. Consequently, at all times of the investigation, including 24 h after immunization, the response of MIF producers was the result of induction of the immune response of these cells.

The results show that MIF producers can be induced *in vivo* by intravenous alloimmunization with minor H antigens, but only if the immune response of these cells was tested in the early stages, and above all, on the 1st day after immunization. The writers showed previously that "early" and "late" MIF producers 1 day old differ in radiosensitivity and in expression of Lyt-antigens [4]. Early MIF producers are induced more efficiently than late *in vivo* by mutant H-2K antigens [3]. All these data indicate the existence of a special category of T cells, namely T MIF producing cells, whose immune reponse develops in extremely short times (within 24 h) and which is induced by the widest spectrum of H antigens, both strong and weak. It is perhaps this population which is responsible for MIF production in the early stages of MLC with differences for both H-2 antigens, normal and mutant [6], and for non H-2

antigens [15], and also in the early stages of MLC and growth of tumor cells [7]. Late MIF producers have greater selectivity toward H-2 antigens [3] and are induced only weakly if there are differences for weak histocompatibility antigens. Products of the Mls locus, although inducing the formation of late MIF producers, do so only for a short time; BALB/c cells (Mls^b), moreover, are weaker inducers of the response than DBA/2 (Mls^a) cells, in agreement with results obtained previously for MLC [12].

Minor H antigens induce both the humoral and the cellular immune response badly [1], but early MIF producers were effectively induced by non H-2 antigens even more effectively than by H-2 antigens, in agreement with data obtained in the DTH reaction *in vivo* [13]. MIF producers possibly possess their own spectrum of recognizable antigenic determinants, distinguishing them from other categories of T cells. This hypothesis is supported by results showing differences in immunologic specificity of MIF producers [3]. The fact that minor H antigens, like mutant antigens [8], induce only the early component of the immune response of MIF producers suggests that early MIF producers play a special role of monitoring weak modifications of the organism's own antigens, a role which is close to physiological.

Data on the ability of MIF producers to respond to non H-2 antigens obtained by intravenous immunization *in vivo* in the present investigation agree with the results of an investigation of the response of MIF producers in primary MLC *in vitro* [15]. In a study of the primary MIF production parallel with interferon production and development of the proliferative response it was shown that in certain combinations of responding and stimulating cells, differing for the Mls locus and non H-2 antigens, production of both lymphokines was found in the absence of proliferation. Moreover, if lines differing for non H-2 antigens but identical for the Mls locus were chosen, MIF production *in vitro* was found in the absence of interferon production and development of the proliferative response [15].

MIF production discovered in response to non H-2 antigens may thus be of great biological importance. Some membrane antigens, such as non H-2 or antigens associated with tumors, often cannot induce a considerable proliferative or cytotoxic response of T lymphocytes [10, 11]. Since MIF production by T lymphocytes evidently has a lower threshold of activation [15], it may be an important alternative mechanism of recognition and protection through activation of macrophages and other cellular mechanisms.

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