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Depressed Lymphoproliferative Response in Mixed Leukocyte Reaction After Mls-Locus Alloimmunization

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The proliferation of BALB/C lymphocytes preimmunized with Mls or H-2 incompatible cells in response to alloantigens was studied. The results show that preimmunization with Mls-incompatible spleen cells inhibits the lymphoproliferative response against alloantigens whereas preimmunization with H-2 incompatible spleen cells enhances it. It is suggested that Mls coded determinants activate suppressor mechanisms responsible for the unresponsiveness of these preimmunized lymphocytes against alloantigens.

Key words: Mls-Locus, MLC, Mice.

There are two separate genetic systems in the mouse known to code membrane determinants which are strongly active in mixed lymphocyte cultures (MLC) i. e., lymphocyte-activating determinants (LAD's). One is the major histocompatibility complex (H-2) present in cromosome 17 and the other is the Mls locus (2) (minor mouse lymphocyte stimulating locus) located in chromosome 1 (4). While the H-2 complex was first identified by its serologically detectable determinants and its graft-rejecting properties, the non H-2 Mls locus was first characterized by the marked lymphocyte-activating properties of its determinants in MLC. These LAD's are not serologically detectable and they have been shown to be present in B cells (15) and in macrophages (14) but not in T cells, or erythrocytes (3). Contrary to the H-2 encoded antigens, the Mls-locus encoded determinants are not capable of initiating host versus graft reactions against skin grafts (5), heart grafts (17) and non-haematopoietic cells (9). However the role of the Mls locus determinants in the initiation of graft versus host reactions is well established, as is the H-2 complex (6, 13, 17).

Moreover, the failure of Mls LAD's to generate cytotoxic effectors as measured by the cell-mediated lympholysis test (CML) (1, 11) has been determined,

although the role of the Mls locus responding cells still remains unclear.

In order to further clarify the biological role of the Mls locus reactive cells, the proliferation of lymphocytes from mice preimmunized with Mls locus incompatible cells in response to H-2 or Mls-locus LAD's has been studied.

Materials and Methods

Mice. 2 to 3 month old male mice of the BALB/C (H-2^d; Mls^b) DBA/2 (H-2^d; Mls^a) and CBA/H (H-2^k; Mls^b) strain were use. They came from the London Medical Hospital College and were bred in our Laboratory.

Culture Medium. All cultures were performed in RPMI (DIFCO) containing added ampicillin (100 μg/ml), cloxacillin (100 μg/ml) and gentamycin (5 μg/ml), foetal calf serum (Difco) previously heat inactivated to a final concentration of 10 % and glutamine (DIFCO 2 mM). The pH was adjusted between 7.2-7.4 with 4.4 % sodium bicarbonate (Wellcome).

Experimental procedures. BALB/C mice were inoculated i. p. with 10 spleen lymphocytes from, *a*) DBA/2 (H-2 identical; Mls incompatible), *b*) CBA/H (H-2 incompatible; Mls identical) and *c*) BALB/C (singenic cells). Ten days later the animals were killed by cervical dislocation, their lymph nodes (axillary and mesenteric) removed and the lymphoproliferative response in mixed leukocyte cultures was tested.

Mixed leukocytes cultures. In our mixed leukocyte cultures, peritoneal adherent cells (macrophages) were used as stimulating cells since these do not proliferate and since LAD's of the H-2 complex as well as LAD's of the Mls-locus are strongly expressed on the cells (14), as has already been described.

These macrophages were obtained by i.p. injections with 4 ml of the above described culture medium from the peritoneum of DBA/2, CBA/H or BALB/C mice. After massage of the abdomen, the medium was removed and the macrophage concentration adjusted to 2.5 10^5/ml. 200 μl of this cell suspension was added to Nunc Microtiter plates. After 2 h of incubation (37° C, 5 % CO_2) the adherent cells were washed twice with medium and the macrophage monolayer used as stimulating cells. The responder lymphocytes were obtained from the lymph nodes removed from the preimmunized BALB/C mice. They were gently pushed through a mesh screen to produce a single cell suspension and were washed twice in the medium. The lymphocyte suspension was adjusted to 10^6/ml and 200 μl were added to the macrophage monolayer. The cells were incubated for 96 h, then 1 μCi of 3-H-Thymidine (20 μCi/mmol) was added to each cell and further incubated for another 16 h. They were then recollected with a semiautomatic harvester and counted in a LKB 1215 scintillation counter.

Results

MLC response of BALB/C lymphocytes after Mls-locus alloimmunization. 3-H-Thymidine uptake by BALB/C lymphocytes in response to H-2 or Mls-locus encoded LAD's is markedly depressed when the responder lymphocytes come from BALB/C mice preimmunized with DBA/2 (Mls incompatible) spleen cells, when compared with control spleen cells (injected with BALB/C singenics cells). Thus the stimulation index (S. I.) observed in Mls-locus preimmunized lymphocytes in response to DBA/2 macrophages (Mls incompatible) is 0.9 ± 0.2 while the S. I. developed by control lymphocytes is 2.5 ± 0.8. When CBA/H macrophages (H-2 incompatible) are used

Table I. *^{3}H-thymidine uptake by lymph-node lymphocytes from BALB/C mice preinoculated with 10^7 spleen cells from BALB/C (Control group) or DBA/2 (Mls incompatible) mice or CBA/H (H-2 incompatible) mice in response to histoincompatible macrophages.*

Responder Lymphocyte	Stimulatory Macrophage	Incompatibility	c.p.m.	S.I.
BALB/C anti BALB/C (Singenic)	BALB/C	—	2593 ± 658	—
	CBA/H	H-2	9029 ± 1592	3.7 ± 1.3
	DBA/2	Mls	6418 ± 860	2.5 ± 0.8
BALB/C anti DBA/2 (Mls-incompatibility)	BALB/C	—	2880 ± 671	—
	CBA/H	H-2	5467 ± 1363**	1.8 ± 0.3
	DBA/2	Mls	2825 ± 897*	0.9 ± 0.2
BALB/C anti BALB/C (Singenic)	BALB/C	—	1572 ± 311	—
	CBA/H	H-2	4260 ± 600	2.8 ± 0.6
	DBA/2	Mls	3693 ± 891	2.5 ± 0.7
BALB/C anti CBA/H (H-2 incompatibility)	BALB/C	—	1824 ± 335	—
	CBA/H	H-2	7213 ± 1133*	4.0 ± 1.0
	DBA/2	Mls	6698 ± 664*	3.7 ± 0.5

* $p < 0.001$; ** $p < 0.01$

as stimulating cells a similar inhibition in the MLC response of Mls preimmunized animals (1.8 ± 0.3) is observed as compared with control (3.7 ± 1.3) (table I).

MLC response of BALB/C lymphocytes after H-2 alloimmunization. 3-H-Thymidine uptake by BALB/C lymphocytes in response to H-2 Mls codified LAD's appears enhanced when the lymphocytes come from BALB/C mice preimmunized with CBA/H (H-2 incompatible) spleen cells, when compared with controls injected with singeneic BALB/C spleen cells. Thus the S.I. observed in H-2 preimmunized lymphocytes in response to CBA/H macrophages (H-2 incompatible) is 4.0 ± 0.1 while the control lymphocytes S.I. is 2.8 ± 0.6. A similar enhancement is also observed when DBA/2 macrophages (Mls incompatible) are used as modulators in the MLC, being the H-2 preimmunized lymphocytes S. I. 3.7 ± 0.5 and the control lymphocytes S.I. 2.5 ± 0.7.

The H-2 or Mls-Locus preimmunization does not affect the basal 3-H-Thymidine uptake of the BALB/C lymphocytes incubated with singeneic macrophages (table I).

Discussion

The results indicate that immunization with Mls incompatible cells not only does not induce an enhancement of the proliferative response of these lymphocytes when they are stimulated with Mls-incompatible cells but it induces suppression of the response. This suppression is non antigen specific since the anti H-2 response is also markedly depressed.

However, H-2 preimmunization induces an enhancement of the proliferative response against H-2 of Mls locus codified LAD's. These results may be explained according to PLA and COLOMBANI (10) by the potentiation of MLC against different antigens observed when the responder lymphocytes were previously sensitized against a histocompatible antigen.

On the other hand, the suppressed

MLC developed by Mls-locus preimmunized lymphocytes stimulated by H-2 antigens could explain the inhibition of cell mediated lympholysis against (H-2) allospecificities observed after inoculation of Mls incompatible cells (8) since in the generation of cytotoxic T cells a previous precursor's proliferation is required (16). Although this suppression could be explained by a preemption process of H-2 responding clones (8) it can be inferred that preimmunization with Mls incompatible cells induces the proliferation of suppressor cells (7) that could modulate the immune response inhibiting T cell proliferation by acting either through cell contact with the potentially activated clone or through the release of a factor that suppresses its activation. Thus, instead of cooperating with prekiller cells to produce effectors against H-2 antigens, the Mls-reactive cells would interact with these cytotoxic precursors, inactivating them and preventing them from proliferation and generation of cytotoxic effectors. However, further studies are necessary to clarify the intrinsic cellular and biochemical mechanisms by which Mls preimmunized lymphocytes have a depressed proliferative response when stimulated by H-2 of Mls-locus LAD's in mixed leukocyte cultures.

Resumen

Se estudia la capacidad de los linfocitos de ratones BALB/C inyectados con células incompatibles en el complejo H-2 o en el locus Mls para proliferar al ser estimuladas por aloantígenos. Los linfocitos inmunizados con células incompatibles en el locus Mls, muestran una inhibición en su respuesta a los aloantígenos, mientras que los procedentes de ratones inmunizados con células incompatibles en el complejo H-2 muestran una potenciación de la respuesta. Estos resultados indican que la inmunización con células incompatibles en el Mls pone en marcha un fenómeno de supresión de la respuesta inmune celular.

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