The Evaluation of Biologically Active Supernatants of Cultures of Lymphoid Cells from Rabbits with Delayed (Cellular) Hypersensitivity on a Macrophage Layer

Following the interaction of hypersensitive lymphoid cells with the antigen biologically active substances are released[1,2]. Their activities are tested mostly by means of migration inhibiting test[3–5].

Recently some authors[6,7] studied the reaction of isolated peritoneal exudate cells from delayed hypersensitive animals with the specific antigen by means of cytological methods and observed the disappearance mainly of spreading forms. BARNET[8–10], using a modified cultivation chamber, demonstrated in addition to these morphological changes an alteration of the vitality of the cells.

In the present work the method using layers of peritoneal macrophages from normal non-sensitized rabbits[9] was used for the evaluation of the above-mentioned biologically active substances.

The preparation of the biologically active supernatants, as well as their testing by migration inhibitory test, were described previously[11].

Control non-influenced cultures are characterized by macrophage monolayers consisting of well spreading active macrophages, some of which form pseudopodia (Figure 1). The appearance of this type of culture does not substantially change during the 2 and 20 h of cultiva-

Table 1. Evaluation of the effect of supernatants A and B on the layer of normal peritoneal macrophages

a) After 2 h of cultivation

Supernatant	Experiment No.																											
	I			II			III			IV			V			VI			VII			VIII			IX		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
A 1:1	356	28	0.88			0.88			0.88			0.88			0.88			0.85			0.85			0.55			0.55
1:2							204	17		289	27					200	39		320	29		211	15		304	11	
1:4																319	29					240	36		315	20	
1:10	406	24		440	25		249	17		236	25					312	28		340	24		216	23		328	26	
1:50				415	20		219	24		281	23					290	30		326	24		265	24		330	26	
B 1:1	484	2	0.49			0.49			0.47			0.47			0.47			0.47			0.47			0.52			0.52
1:2							222	4		297	3					230	2		368	2		195	2		319	4	
1:4																233	5					267	10		307	6	
1:10	418	8		457	2		183	7		264	10		269	4		342	4		365	3		260	6		383	7	
1:50				433	1		215	15		249	14		300	8		285	4		377	5		297	11		335	14	
1:250													346	11					395	12							
PPD tuberculin 1 µg/ml				445	20	0.97																					
PPD tuberculin 5 µg/ml				437	16	0.95																					
Control	448	35	1.0	419	14	1.0	208	24	1.0	332	31	1.0	361	22	1.0	324	39	1.0	338	23	1.0	195	26	1.0	329	33	1.0

b) After 20 h of cultivation.

Supernatant	VI			VII			VIII			IX		
	a	b	d	a	b	d	a	b	d	a	b	d
A 1:2	111	13		227	3		158	9	99	229	7	68
1:4	133	24		265	16		228	26	18	316	32	3
1:10	192	32		329	22		284	22	23	349	32	8
1:50	197	42		250	31		312	25	12	337	26	15
1:250				329	25							
B 1:2	109	2		243	3		210	2	99	278	0	26
1:4	95	3		196	3		262	2	64	321	2	11
1:10	149	6		271	6		316	9	26	359	8	8
1:50	190	13		255	7		334	13	20	345	11	9
1:250				309	19							
PPD tuberculin 10 µg/ml				290	25							
Control	207	34		296	23		266	17	24			

Supernatant A, control supernatant of a culture of hypersensitive lymph node cells without antigen. Supernatant B, active supernatant of a culture of hypersensitive lymph node cells with specific antigen – PPD tuberculin. a, number of adhering cells counted in 3 random fields (magnif. ×600) representing only a sample of the whole macrophage layer. b, percentage of cells possessing cytoplasmic protrusions (motile fibroblastoid forms). c, migration inhibiting activity expressed in cytotoxic indices. d, percentage of eosin stained cells.

Fig. 1. Control noninfluenced culture of macrophage monolayer with spreading active macrophages forming pseudopodia (2 h cultivation). Stained with May Grünwald Giemsa. ×600.

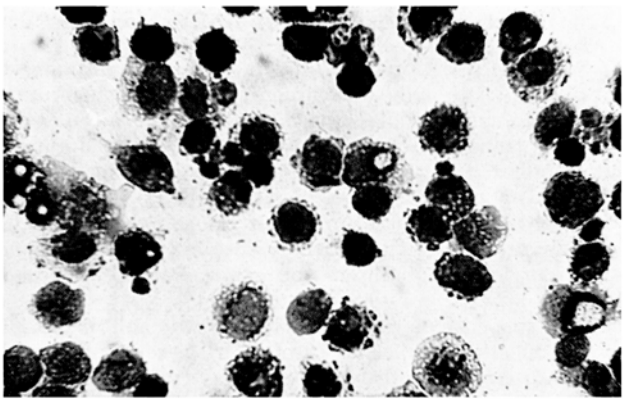

Fig. 2. Culture of macrophage monolayer cultivated with supernatant B; prevalence of round cells lacking the pseudopodia (2 h cultivation). Stained with May Grünwald Giemsa. ×600.

tion. The specific effect of the active supernatant (Sup. B), i.e. from the cultivation of hypersensitive lymphoid cells with antigen, was on the contrary well marked after 2 h of cultivation. The culture as a whole had an appearance of a non-active, inhibited culture characterized by prevalence of round cells lacking the pseudopodia, with diminished number of motile fibroblastoid forms (Figure 2). The inhibitory effect of the supernatant B is well marked even after further dilution (Table). The addition of a control supernatant (A), i.e. from the cultivation without antigen, did not influence, in most cases, the appearence of macrophage culture so that it does not differ from the control cultivations. The antigen (PPD tuberculin) had also no inhibitory effect.

At the 20 h cultivation period, the vital staining as a further criterion was included with the assumption that the cytotoxic effect could also be tested. The results given in the table show, however, that the best parameter for the evaluation of the activity of the supernatants remains still the diminution of the number (or perhaps the disappearance) of the motile forms. This criterion is regularly positive in the active supernatants (B), even in higher dilutions. A marked cytotoxic effect characterized by heightened number of eosin-stained cells could be observed regularly in the active supernatant B and in some batches of supernatant A (in the dilution 1:2 only).

The comparison of the experimental results with the parallelly performed migration inhibitory tests (Table) shows that the effect of the biologically active substances on the macrophage motility seems to be in correlation with its migration inhibiting capacity. These observations represent a further proof for the assumption that the mechanism of the migration inhibition of macrophages is due principally to the inhibition of the pseudopodia formation[6, 7, 10].

We suppose, therefore, that the method can be used as a complementary one for the evaluation of the biologically active substances produced and liberated following the interaction of the hypersensitive lymphoid cells with the specific antigen.

The advantage of the above-mentioned method is the possibility of following simultaneously 2 parametres, i.e. the inhibition of pseudopodia formation and the changes in cell vitality, which enables us to detect either different degrees or distinct biological activities i.e. MIF and the lymphotoxin[1].

On the other hand, there may be disadvantages in the inability to obtain a standard macrophage monolayer which may cause the absolute values of the substances tested to vary from experiment to experiment (due to the quality of macrophages used).

Even though the parameters followed enable a semiquantitative evaluation, they provide ofcourse only a partial impression of the whole picture of the culture. The total view, which is ofcourse still more impressive, could provide a better evaluation but it can hardly be presented in a table.

The final effect, based especially on the comparison with the control cultivations, gives reproducible and valuable results, however.

Zusammenfassung. Supernatanten aus Kulturen überempfindlicher Lymphozyten und dem spezifischen Antigen mit MIF-Aktivität können die Entstehung der Pseudopodien normaler Makrophagen verhindern und zeigen nach längerem Kultivieren zuweilen toxische Eigenschaften.

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