ACTA VET. BRNO, 58, 1989: 281–296

THE USE OF MOUSE MODEL FOR THE DETERMINATION OF PROTECTIVE ACTIVITY IN SALMONELLA-SPECIFIC LEUCOCYTE DIALYZATE

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Received June 6, 1988

Abstract

Mikula I., Pistl J.: *The Use of Mouse Model for the Determination of Protective Activity in Salmonella-specific Leucocyte Dialyzate.* Acta vet. Brno 58, 1989: 281–296.

The specific Leucocyte dialyzate (DLE^s) was prepared from the peripheral blood leucocytes, mesenteric lymph nodes and spleens of calves that have been vaccinated and subsequently infected with virulent *S. typhimurium* strain. The non-specific dialyzate (DLE^n) was obtained from the lymph nodes of fattened bulls. The inhibition of *Salmonella* penetration into the liver and spleen as well as the colonization of digestive tract were tested in SPF white mice and C57BL/6 inbred mice to which DLE was administered and then were infected with *S. typhimurium* strain. The application of DLE^s induced a marked inhibition and/or elimination of penetrative abilities of virulent *S. typhimurium* strain in white mice. In C57BL/6 inbred mouse line, DLE partially inhibited multiplication of salmonellae in the liver and spleen, respectively. DLE^n did not inhibit the penetration and colonization of salmonellae.

The standardization of DLE preparations was carried out by measuring of optical density at 260 nm (OD_{260}). The solution of specific DLE at OD_{260} of 1.5 (10-fold concentrated) inhibited and/or eliminated the penetration and colonization properties of *S. typhimurium.*

The fractionation of DLE^s through Sephadex G-25 confirmed heterogeneity of fractions in the protection against salmonellosis. The index ratio of OD_{260} to OD_{280} as tested for Sephadex fractions showed different values than in the case of DLE preparation.

Leucocyte dialyzate (DLE), Salmonella typhimurium, immunity, mice.

Dialyzable leucocyte extract (DLE) characterized as a preparation obtained by disruption of leucocytes (concentrated in "buffy coat") can also be isolated from the lymph nodes and spleens and after dialysis DLE contains low-molecular components with a great part of them formed for example by thymosin (Wilson 1983). The compound from DLE with molecular weight of approximately 3 500 daltons presents

antigen-specific transfer factor (TF) (Fudenberg 1986). In the present, the term "transfer factor" is used for dialyzable low-molecular leucocyte components that can mediate the T-lymphocyte response of antigen-specific nature (Wilson and Fudenberg 1983). The enzymatic studies characterized TF as complete in vivo molecules composed of RNA base and peptides. The disruption of these molecules resulted in the loss of biological activity (Wilson et al. 1976).

DLE is able to transfer not only positivity in the skin test but is also responsible for the production or initiation of other reactions of cell-mediated immunity in various immunodeficient conditions (Levin 1970). Immunity induced with DLE is characterized by production of migration-inhibition factor (MIF), by stimulation of macrophages and lymphocytes.

The unit for testing of DLE efficiency is defined as amount of DLE obtained from 5×10^8 leucocytes (Khan et al. 1979). The potency unit is defined as DLE amount which can induce 20% antigen-specific inhibition of leucocyte migration (Fudenberg 1980; Wilson et al. 1982). The other methods for *in vitro* testing of DLE efficacy are as follows: transformation of lymphocytes (Ablin 1980), E-rosette test, tests for phagocyte activity and chemotaxis activity (Arala-Chaves et al. 1977).

In this work we present a method for testing of DLE^s efficiency on mouse model enabling to characterize a minimum inhibition dose of *Salmonella*-specific DLE^s and protective activity in Sephadex fractions as well.

Materials and Methods

Experimental animals

Conventional white SPF mice and C57BL/6 mouse inbred line (VELAZ, ÚSOL-Praha) were used in our experiments.

Dialyzable leucocyte extract

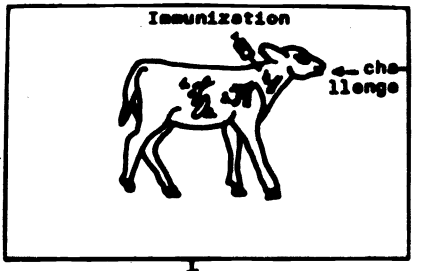
a) DLE^s was prepared from the peripheral blood leucocytes (concentrated in "buffy coat"), mesenteric lymph nodes and spleens of calves immunized against salmonelosis with "Salinvak" vaccine (made in Czechoslovakia) and subsequently infected with S. typhimurium (Fig. 1). Lymph nodes and spleens following homogenization and 10-fold cryolysis were centrifuged at 10 000 m. $s.^{-2}$. Supernatant was subjected to filtration through whirling asbestic-cellulose filter (Seitz K3) and filtrate was dialyzed against distilled water containing 0.1% maltose at 4° C. Dialyzates concentrated by freeze-drying were resuspended and purified through Amicon apparatus fitted with UM5 membrane. As for testing of efficient dose, DLE was diluted to the values of 0.35, 0.6, 0.8, 1.0 and 1.5 at OD_{260} as well as for 5-, 10-, 50- and 100-fold values at OD_{260} that equals to 1.5. DLE determined at OD_{280} corresponded to 0.8 – 1.0 values.

b) Non-specific DLE was prepared from the bull's lymph nodes and spleens. Fattening bulls were immunized by a mixture of viral and bacterial antigens used for the production of serobronchin (product made by Bioveta, Nitra).

Gel chromatography

Fractionation of DLE was carried out through Sephadex G-25 column (2×45 cm) eluted with Tris-HCl buffer (pH 7.2) at a flow rate of 0.1 ml $min.^{-1}$ and 4° C. 100-fold concentrated sample of 3 ml volume was logded on column (original $OD_{260} = 1.8$). The individual 3 ml fractions were collected and following OD_{260} and OD_{280} measurements were stored in frozen condition until further testing.

PREPARATION OF LEUCOCYTE DIALYSATE



YIELDING OF BIOLOGICAL MATERIAL

1. Mesenteric lymph nodes
2. Mediastinal lymph nodes
3. SPLEEN
4. Peripheral blood

P R O C E S S I N G

A. HOMOGENIZATION OF TISSUES (1–3)
- Freezing and thawing cycle
- Centrifugation

B. SEPARATION AND ISOLATION OF LYMPHOCYTES (4)
- Filtration of supernatant
- Dialysis of filtrate against distilled water
- Concentration
- Lymphocyte dialysate (transfer factor)
- Filtration through antibacterial filter

} APPLICATION OF PREPARATION i.p.

Fig. 1

DLE application

DLE measured at the respective OD_{260} concentration as well as the fractions selected from the gel chromatography were filtrated through antibacterial filter G5 and then applied intraperitoneally to mice in volume of 0.5 ml.

Infection of mice

Mice were orally infected with *S. typhimurium* 4/5 strain at a dose of 10^3 CFU (colony forming units).

Testing for DLE efficiency

10-fold concentrated DLE (OD_{260} = 1.8) was i.p. administered to mice (Tab. 1). Mice in the total number of 220 were divided into 6 groups. Mice from the 1st, 3rd and 5th groups following DLE application were infected with *S. typhimurium* 4/5 after 24 hours. Mice out of the 2nd, 4th and 6th groups were infected on day 7 after DLE administration. DLE^s was given to mice of the 1st up to 4th groups and DLE^n

TESTING OF LeD ON MICE

GROUP OF MICE	PARAMETERS OF TEST									
	LeD		NUMBER OF MICE				ORAL INFECTION S. TYPHIMURIUM 4/5		KILLING OF MICE POSTINFECTION	
	DOSE/MOUSE (ml)	APPLICATION	SPECIFIC LeD	C	NON-SPECIFIC LeD	C	DOSE/0,1 ml (CFU)	DAY AFTER LeD APPLICATION	DAYS	SAMPLES COLLECTED
1/A WHITE SPF	0,5	i.p.	20	-	-	-	10^3	1	3,6,9,30	LIVER SPLEEN GUT
K WHITE SPF	Placebo 0,5		-	20	-	-	10^3	1		
2/A WHITE SPF	0,5		20	-	-	-	10^3	7		
K WHITE SPF	Placebo 0,5		-	20	-	-	10^3	7		
3/A C57BL/6	0,5	i.p.	20	-	-	-	10^3	1	3,6,9,30	LIVER SPLEEN GUT
K C57BL/6	Placebo 0,5		-	20	-	-	10^3	1		
4/A C57BL/6	0,5		20	-	-	-	10^3	7		
K C57BL/6	Placebo 0,5		-	20	-	-	10^3	7		
5/A WHITE SPF	0,5	i.p.	-	-	18	-	10^3	1	3,6,9	LIVER SPLEEN GUT
K WHITE SPF	Placebo 0,5		-	-	-	18	10^3	1		
6/A WHITE SPF	0,5		-	-	18	-	10^3	7		
K WHITE SPF	Placebo 0,5		-	-	-	18	10^3	7		

LEGEND : LeD = LEUCOCYTE DIALYSATE; CFU = COLONY FORMING UNITS; A = APPLICATION; C = CONTROL

Tab. 1

to mice of the 5th and 6th groups. Ten animals from each group (5 experimental and 5 controls) were killed on days 3, 6, 9 and 30 post-infection.

Testing for protective activity

Testing for the minimum protective dose of DLE was done in 11 groups of mice (Tab. 2). The tests of protective activity for the individual fractions of *Salmonella*-specific DLE were carried out in 24 groups of mice (Tab. 3). Twenty three representative fractions were selected for intraperitoneal inoculation of mice based upon

DETERMINATION OF MINIMUM PROTECTIVE DOSE FOR SPECIFIC LcD

PARAMETERS OF TEST		GROUPS OF MICE										
		I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	C
NUMBER OF MICE IN GROUP		10										10
LcD	DOSE/MOUSE	0,5 ml										-
	ROUTE OF APPLICATION	INTRAPERITONEALLY										
	OD_{260}	0,35	0,6	0,8	1,0	1,3	1,5	1,5	1,5	1,5	1,5	-
	CONCENTRATION	1	1	1	1	1	1	5 FOLD	10FOLD	50FOLD	100FOLD	-
INFECTION WITH S.TYPHIMURIUM 4/5	DOSE/MOUSE(CFU)	10^3										
	ROUTE OF APPLICATION	ORAL										
	TIME OF INFECTION	ON DAY 4 AFTER LcD APPLICATION										
TIME OF SAMPLING		ON DAY 8 AFTER ORAL S.TYPHIMURIUM INFECTION										
SELECTED SAMPLES		LIVER, SPLEEN, GUT										
EVALUATION OF TEST		ESTIMATION OF SALMONELLA CFU IN ORGANS										

LEGENDA: C = CONTROL GROUP
LcD = LEUCOCYTE DIALYSATE
OD = OPTICAL DENSITY

Tab. 2

ESTIMATION OF SALMONELLA-SPECIFIC LcD PROTECTIVE ACTIVITY IN SEPHADEX FRACTIONS

PARAMETERS OF TEST		FRACTION No.																							
		9	20	23	25	28	30	32	33	35	37	39	43	45	49	51	55	56	57	58	59	60	61	63	C
NUMBER OF MICE IN GROUP		6																							6
LcD	DOSE/MOUSE	0,5 ml																							-
	ROUTE OF APPLICATION																								-
	OD_{260}	0,09	0,08	0,67	0,96	0,99	1,08	1,16	1,23	1,45	1,68	1,37	0,62	0,40	0,33	0,34	0,26	0,60	0,60	0,50	0,53	0,42	0,23	0,02	-
	OD_{280}	0,02	0,03	0,20	0,41	0,43	0,50	0,55	0,80	0,66	0,74	0,66	0,41	0,51	0,84	0,85	0,78	1,82	1,70	1,90	1,85	1,34	1,83	0,09	-
INFECTION WITH S.TYPHIMURIUM 4/5	DOSE/MOUSE	10^3																							
	ROUTE OF APPLICATION	ORAL																							
	TIME OF INFECTION	ON DAY 4 AFTER LcD APPLICATION																							
TIME OF SAMPLING		ON DAY 8 AFTER S.TYPHIMURIUM INFECTION																							
SELECTED SAMPLES		LIVER, SPLEEN, GUT																							
EVALUATION OF TEST		ESTIMATION OF SALMONELLA CFU IN ORGANS																							

LEGENDA: C = CONTROL GROUP
LcD = LEUCOCYTE DIALYSATE
OD = OPTICAL DENSITY

Tab. 3

ELIMINATION OF COLONIZATION AND PENETRATION OF S.TYPHIMURIUM STRAIN 4/5 BY SALMONELLA-SPECIFIC LcD AS TESTED ON CONVENTIONAL WHITE SPF MICE

DAY OF KILLING	ORGAN	GROUP 1			GROUP 2		
		A	C	T – TEST	A	C	T – TEST
		INFECTION^{x} AT 24 h AFTER LcD APPLICATION	INFECTION^{x} AT 24h AFTER PLACEBO APPLICATION		INFECTION^{x} ON DAY 7 AFTER LcD APPLICATION	INFECTION^{x} ON DAY 7 AFTER PLACEBO APPLICATION	
		$\bar{x} \pm$ sd (log)	$\bar{x} \pm$ sd (log)	SIGNIFICANCE	$\bar{x} \pm$ sd (log)	$\bar{x} \pm$ sd (log)	SIGNIFICANCE
3	L	ß	ß	ß	ß	ß	ß
	S	ß	ß	ß	ß	ß	ß
	G	3,11 ± 1,3	4,96 ± 0,93	+	ß	4,32 ± 0,82	++++
6	L	ß	5,94 ± 0,89	++++	ß	5,34 ± 0,75	++++
	S	ß	5,84 ± 0,78	++++	ß	4,96 ± 0,92	++++
	G	4,04 ± 0,5	7,24 ± 0,16	+++	2,70 ± 0,77	7,48 ± 0,56	+++
9	L	ß	5,49 ± 0,96	++++	ß	5,27 ± 0,73	++++
	S	ß	4,94 ± 1,21	++++	ß	4,39 ± 0,87	++++
	G	3,03 ± 1,7	6,36 ± 0,84	++	1,17 ± 1,13	5,83 ± 0,66	+++
30	L	ß	1,62 ± 1,4	++++	ß	2,16 ± 0,82	++++
	S	ß	0,89 ± 0,69	++++	ß	1,14 ± 0,75	++++
	G	1,71 ± 1,04	4,09 ± 1,48	+	ß	3,78 ± 0,85	++++

LEGEND : STATISTICAL SIGNIFICANT DIFFERENCES BETWEEN CONTROL AND EXPERIMENTAL GROUPS: +p<0.05., ++p<0,01., +++p<0.001., ++++ = POSITIVE VALUE OF CONTROL IN COMPARISON TO NEGATIVE FINDING OF EXPERIMENTAL GROUP., L = LIVER., S = SPLEEN., G = GUT., $\bar{x} \pm$ sd = AVERAGE log VALUE OF CFU SALMONELLA ± DEVIATION., x = INFECTION p.o. AT A DOSE OF 10^3 CFU. LcD = LEUCOCYTE DIALYSATE., A = APPLICATION., C = CONTROL.

Tab. 4

OD_{260} and OD_{280} (Fig. 2). After DLE application (0.5 ml dose) mice were infected with *S. typhimurium* on day 4. On day 8 post-infection mice were killed and necropsied. The difference in CFU between the experimental and control groups of mice allowed to calculate the inhibition of penetration and colonization of *S. typhimurium* strain used for challenge.

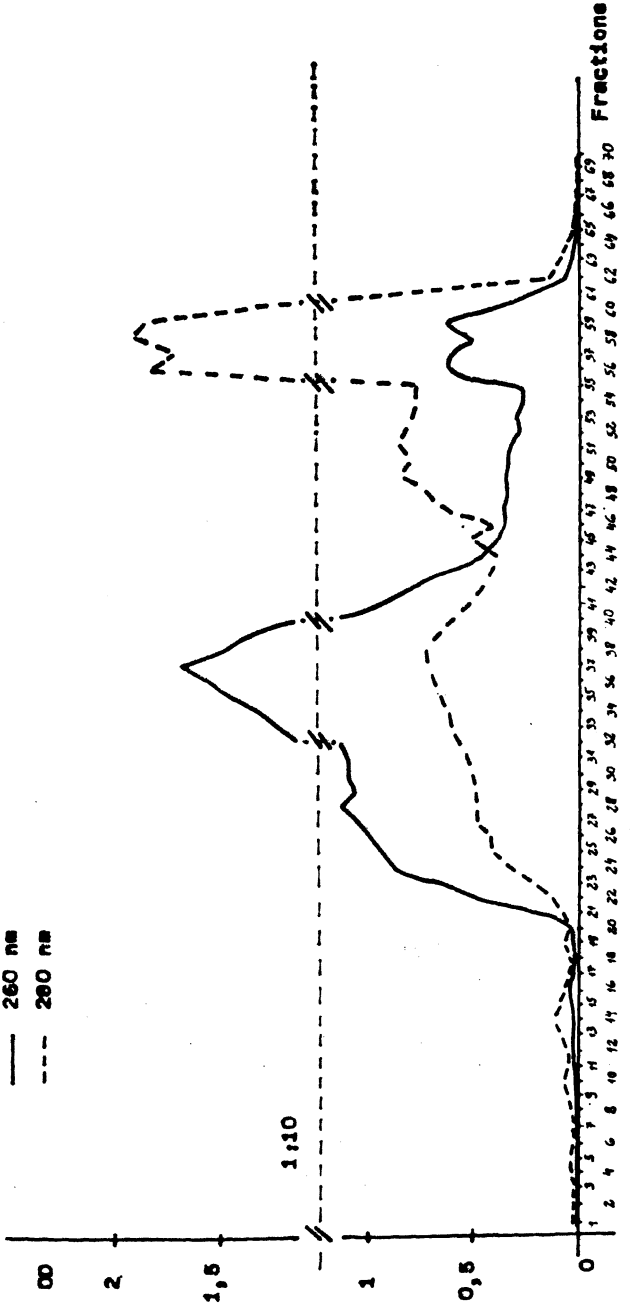
Evaluation of the test

Mice were killed and immediately subjected to necropsy. The bacterial counts of *Salmonella* (CFU) were determined in the parenchymatous organs and gut, respectively. The single counts of *Salmonella* CFU were calculated for 1 g of biological material, converted to logarithms and the mean value ($\bar{x}$) and the standard deviation ($\pm$ sd) were determined. Results were evaluated using Student's t-test.

Results

A. Testing for DLE^{s} efficiency in conventional SPF white mice (Tab.4)

Mice from the 1st group were infected with virulent *S. typhimurium* strain 24 h after DLE application. The 2nd group of mice was infected on day 7. Mice of both groups were killed on days 3, 6, 9 and 30 from the time of infection. There was significant difference in the counts of *Salmonella* CFU present in the gastrointestinal tract of the 1st group of mice on day 3 post-infection as compared to controls ($p < < 0.05$). The presence of salmonellae has not been quantitatively detected in the parenchymatous organs and gut in the 2nd group of mice.



LEGENDA: LcD = 100-FOLD CONCENTRATION AT OD_{260} 1,5

Fig. 2

On day 6 post-infection, salmonellae were not present in the parenchymatous organs of mice of the 1st and 2nd groups while the counts of salmonellae in controls expressed by a value of average logarithm were 5.94 and 5.34 in the liver and 5.54 and 4.95 in the spleen. In both control groups there was statistically significant difference in the counts of *Salmonella* CFU present in the gut ($p < 0.001$). Similar results were found on day 9 post-infection indicating the significant difference in the penetration of *Salmonella* into the parenchymatous organs of mice to which specific DLE was applied.

B. Testing for DLE^s efficiency on C57BL/6 inbred mouse line (Tab. 5).

Testing of DLE^s was performed on C57BL/6 inbred mouse line that is highly susceptible to *Salmonella* infection because of the defect in Ity gene.

The colonization and penetration of salmonellae were found in the control group of mice on day 3 post-infecti on. The penetration of *Salmonella* into the parenchymatous organs has been significantly reduced as determined by the counts of *Salmonella* CFU in mice of the 3rd and 4th groups ($p < 0.001$). The significant values were also recorded for *Salmonella* CFU in the gut ($p < 0.01$ in group 3, and $p < 0.01$ in group 4, respectively). On day 6 post-infection there were significant differences in the counts of CFU in the 3rd group of mice only in the parenchymatous organs ($p < 0.05$ in the liver and $p < 0.01$ in the spleen) and in the same organs in group 4($p < 0.001$).

The significant differences have not been found in the counts of *Salmonella* CFU in the 3rd group of mice on days 9 and 30. In group 4 of mice there were significant differences in the liver ($p < 0.05$) and spleen ($p < 0.01$) on day 9 and also in the liver ($p < 0.01$), spleen ($p < 0.05$) and gut ($p < 0.05$) on day 30, respectively.

ELIMINATION OF COLONIZATION AND PENETRATION OF S.TYPHIMURIUM STRAIN 4/8 BY SALMONELLA-SPECIFIC LeD AS TESTED ON INBRED C57BL/6 MOUSE LINE

DAY OF KILLING	ORGAN	GROUP 3			GROUP 4		
		A	C	T – TEST	A	C	T – TEST
		INFECTION^x AT 24 h AFTER LeD APPLICATION	INFECTION^x AT 24 h AFTER PLACEBO APPLICATION		INFECTION^x ON DAY 7 AFTER LeD APPLICATION	INFECTION^x ON DAY 7 AFTER PLACEBO APPLICATION	
		$\bar{x} \pm sd$ (log)	$\bar{x} \pm sd$ (log)	SIGNIFICANCE	$\bar{x} \pm sd$ (log)	$\bar{x} \pm sd$ (log)	SIGNIFICANCE
3	L	0,38 ± 0,80	5,87 ± 0,48	+++	β	4,34 ± 0,75	++++
	S	β	5,89 ± 0,71	++++	0,66 ± 0,92	5,20 ± 0,43	+++
	G	4,17 ± 1,27	6,79 ± 0,76	++	4,04 ± 0,43	6,45 ± 0,82	+++
6	L	3,63 ± 0,85	5,04 ± 0,48	+	2,36 ± 0,47	5,14 ± 0,56	+++
	S	2,79 ± 0,89	4,37 ± 0,39	++	0,98 ± 1,34	3,86 ± 0,44	+++
	G	5,32 ± 1,01	4,84 ± 1,60	β	3,97 ± 0,47	4,17 ± 0,92	β
9	L	6,40 ± 1,41	6,88 ± 0,86	β	5,76 ± 0,68	6,74 ± 0,48	+
	S	5,76 ± 1,12	6,66 ± 0,42	β	4,46 ± 0,47	6,36 ± 0,32	++
	G	6,44 ± 0,24	6,91 ± 0,73	β	6,47 ± 0,85	6,60 ± 1,30	β
30	L	3,63 ± 1,71	3,10 ± 0,34	β	1,69 ± 0,48	3,28 ± 0,56	++
	S	3,13 ± 1,99	2,30 ± 0,60	β	0,95 ± 0,88	2,69 ± 0,38	+
	G	5,13 ± 1,68	4,84 ± 1,18	β	2,88 ± 0,35	4,66 ± 0,89	+

LEGEND : STATISTICAL SIGNIFICANT DIFFERENCES BETWEEN CONTROL AND EXPERIMENTAL GROUPS: +p<0.05., ++p<0,01., +++p<0.001., ++++ = POSITIVE VALUE OF CONTROL IN COMPARISON TO NEGATIVE FINDING OF EXPERIMENTAL GROUP., L = LIVER., S = SPLEEN., G = GUT., $\bar{x} \pm sd$ = AVERAGE log VALUE OF CFU SALMONELLA ± DEVIATION., x = INFECTION p.o. AT A DOSE OF 10^3 CFU. LeD = LEUCOCYTE DIALYSATE., A = APPLICATION., C = CONTROL.

Tab. 5

C. Testing for DLE^n efficiency on conventional SPF white mice (Tab. 6)

From the results it followed that non-specific DLE did not inhibit penetration of salmonellae into the parenchymatous organs nor their counts in the gut. There was no statistically significant difference in the counts of *Salmonella* CFU in the single organs of experimental groups of mice in comparison with controls.

ELIMINATION OF COLONIZATION AND PENETRATION OF S.TYPHIMURIUM STRAIN 4/5 BY NON-SPECIFIC LcD AS TESTED ON CONVENTIONAL WHITE SPF MICE

DAY OF KILLING	ORGAN	GROUP B			GROUP B		
		A	C	T – TEST	A	C	T – TEST
		INFECTION^x AT 24 h AFTER LcD APPLICATION	INFECTION^x AT 24 h AFTER PLACEBO APPLICATION		INFECTION^x ON DAY 7 AFTER LcD APPLICATION	INFECTION^x ON DAY 7 AFTER PLACEBO APPLICATION	
		$\bar{x} \pm sd$ (log)	$\bar{x} \pm sd$ (log)	SIGNIFICANCE	$\bar{x} \pm sd$ (log)	$\bar{x} \pm sd$ (log)	SIGNIFICANCE
3	L	1,55 ± 0,39	1,60 ± 0,61	ß	1,95 ± 0,60	1,98 ± 0,44	ß
	S	0,96 ± 0,63	0,64 ± 0,90	ß	1,57 ± 0,99	1,57 ± 1,03	ß
	G	1,95 ± 0,56	2,43 ± 0,57	ß	2,45 ± 0,46	2,47 ± 1,18	ß
6	L	3,35 ± 0,40	3,71 ± 0,45	ß	3,38 ± 0,72	3,78 ± 0,84	ß
	S	2,67 ± 0,80	3,37 ± 0,83	ß	3,17 ± 0,88	3,58 ± 0,63	ß
	G	4,11 ± 0,88	4,47 ± 0,87	ß	3,97 ± 1,16	4,17 ± 1,02	ß
9	L	3,74 ± 0,71	4,44 ± 1,09	ß	3,68 ± 1,68	4,19 ± 1,18	ß
	S	3,73 ± 0,78	4,30 ± 1,11	ß	3,21 ± 1,72	3,76 ± 0,78	ß
	G	4,81 ± 1,27	5,33 ± 1,59	ß	4,57 ± 1,70	4,66 ± 1,60	ß

LEGEND : $\bar{x} \pm sd$ = AVERAGE log VALUE OF SALMONELLA CFU ± DEVIATION
x = INFECTION DOSE OF 10^3 CFU S.TYPHIMURIUM
L = LIVER; S = SPLEEN; G = GUT
LcD = LEUCOCYTE DIALYSATE; A = APPLICATION LcD; C = CONTROL

Tab. 6

D. Testing for minimum protective dose of DLE^s (Tab. 7).

DLE^s in the various concentrations (at required OD_{260}) was administered i.p. to mice. DLE when applied to mice at $OD_{260} = 0.35-1.0$ did not induce any effect in the counts of Salmonella CFU. DLE^s inoculated to mice at $OD_{260} = 1.3$ showed a significant difference in ($p < 0.05$) *Salmonella* CFU in the gut but did not inhibit penetration of *S. typhimurium* strain into the liver and spleen. DLE^s when applied at absorbance of 1.5 has shown a significant difference of CFU in the spleen and liver ($p < 0.05$). 5-fold concentrated DLE^s with $OD_{260} = 1.5$ represented the minimum dose producing the significant difference in all the tested organs. DLE^s doses that were 10-, 50- and 100-fold concentrated have induced significant differences in all tested organs.

E. Testing for protective activity of Salmonella-specific DLE present in Sephadex fractions

The elution profile obtained from 70 fractions of DLE is characterized by means of OD_{260} and OD_{280} absorbances (Fig. 2). Twenty three fractions representing the level of elution curve were assayed for protective activity (Tab. 8).

The most significant inhibition of Salmonella CFU in the liver, spleen and gut ($p < 0.001$) was detected in the fraction no. 58, which was present in the maximum peak of OD_{280}. The inhibition of Salmonella CFU in the liver, spleen and gut was found also in the fractions of maximum OD_{280} peak (fractions nos. 55–61). There were no significant differences in the fractions that showed the minimal and marked absorbance values of maximum OD_{260} peak (fractions nos. 9, 20, 23, 25, 28, 63). The remaining fractions showed a partial inhibition of penetration and colonization of digestive tract as demonstrated in the liver and spleen (fractions nos. 30, 35, 37, 39, 43, 45, 51).

Discussion

The tests for transfer of immunity by means of DLE were done on mouse model by many authors. Li Zailian (1987) applied the porcine specific transfer factor into footpad of mice primed with the viral antigen of encephalitis B. He observed the lymphocyte infiltration in the footpad tissue as compared to the control group. Huang et al. (1987) reported on protective immunity to HSV 1 following specific TF application to BALB/c mouse line. Mayer et al. (1987) studied specificity of cytotoxic cells inducing activity in dialyzate of splenocytes of mice sequentially immunized by three live viruses. After TF application to CBA mice, Krejčí et al. (1987) have found the passive transfer of tolerance to contact sensitivity by transfer factor. In our work we presented results obtained on mouse model for testing of immunity induced by specific DLE in the course of salmonellosis. From the results it followed that our DLE preparation (10-fold concentrated; $OD_{260} = 1.8$ for DLE^s) was able to prevent or markedly reduce the penetration and colonization of salmonellae. The differences observed in the counts of *Salmonella* CFU in the digestive tract of the 1st and 2nd groups and also in the 3rd and 4th groups of mice suggested stronger inhibition of infection on day 7 following specific DLE application. This finding may be associated with potentiation of antigen-dependent DNA synthesis in mature lymphocytes (Arala-Chaves et al. 1976).

Non-specific DLE did not induce any immunity in SPF white mice, i. e. the mouse model represents the sensitive indicator of specificity in the case of *Salmonella* infection, however, it is not applicable for the detection of efficiency in non-specific DLE.

The mechanism of TF effect is indicated by results achieved in C57BL/6 inbred mouse line defective in Ity gene, which results in the dysfunction of the activity of macrophages (Cohen et al. 1976). As it can be seen from the dynamics of *Salmonella* infection, the more rapid development of this disease occurred in inbred line of mice than in conventional white mice. Resuspending of the freeze-dried specific DLE according to OD_{260} allowed determination of its minimum inhibitory concentration inducing such immunity, that eliminated to a significant extent not only the *Salmonella* penetration but also colonization of digestive tract of mice infected with virulent *S. typhimurium* strain. We can recommend, that the minimum inhibitory concentration of DLE that induces statistically significant reduction in the counts of *Salmonella* CFU present in the parenchymatous organs and gut on the level of $p < 0.01$ significance, may be considered the unit of specificity or of activity of the respective substrate.

Mayer et al. (1987) reported on the gel chromatography fractionation of DLE isolated from the spleens of mice immunized with flavivirus. The authors detected tha highest inductive activities of DLE in the 2nd and 3rd peak, respectively. Our results are in agreement with those obtained by Andron and Ascher (1977) who

THE MINIMUM INHIBITION DOSE OF ANTI-SALMONELLA SPECIFIC LEUCOCYTE DIALYSATE

LcD. OD_{260}	ORGAN	THE MEAN CFU OF SALMONELLA $\bar{x} \pm sd$ (log)	T-TEST SIGNIFICANCE OF DIFFERENCE
0,35	L	4,75 ± 0,79	0
	S	4,15 ± 0,96	0
	G	6,63 ± 0,65	0
0,6	L	4,71 ± 0,68	0
	S	3,71 ± 1,06	0
	G	6,33 ± 0,68	0
0,8	L	4,66 ± 0,72	0
	S	4,05 ± 0,78	0
	G	6,18 ± 0,39	0
1,0	L	3,75 ± 0,74	0
	S	3,39 ± 0,60	0
	G	5,61 ± 0,77	0
1,3	L	4,00 ± 0,52	0
	S	3,25 ± 1,01	0
	G	5,34 ± 0,61	+
1,5	L	3,17 ± 0,68	0
	S	2,28 ± 0,80	+
	G	4,98 ± 0,81	+
1,5 5x	L	2,73 ± 0,73	+
	S	1,78 ± 0,73	++
	G	5,16 ± 0,35	++
1,5 10x	L	1,86 ± 1,08	++
	S	1,11 ± 0,96	++
	G	4,40 ± 0,66	++
1,5 50x	L	1,24 ± 1,05	+++
	S	0,62 ± 0,70	+++
	G	4,25 ± 1,01	++
1,5 100x	L	1,27 ± 1,03	+++
	S	0,80 ± 0,64	+++
	G	4,00 ± 0,60	+++
CONTROL	L	4,18 ± 1,15	-
	S	3,63 ± 1,20	-
	G	6,31 ± 0,79	-

LEGENDA: THERE WERE STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN CONTROL AND EXPERIMENTAL GROUP: +$p<0.05$., ++$p<0.01$., +++$p<0.001$. L=LIVER., S=SPLEEN., G=GUT. $\bar{x} \pm sd$ = THE MEAN log VALUE OF SALMONELLA CFU ± DEVIATION.

Tab. 7

THE PROTECTIVE ACTIVITY OF SEPHADEX FRACTIONS OBTAINED FROM SALMONELLA-SPECIFIC LcD

FRACTION No.	THE MEAN CFU OF SALMONELLA $\bar{x} \pm sd$ (log)			T - TEST SIGNIFICANCE OF DIFFERENCE		
	L	S	G	L p	S p	G p
9	3,13 ± 1,20	2,68 ± 1,17	5,48 ± 1,07	0	0	0
20	3,45 ± 0,94	2,90 ± 0,57	5,50 ± 0,80	0	0	0
23	3,25 ± 1,17	3,08 ± 0,77	5,29 ± 1,50	0	0	0
25	3,33 ± 0,60	3,11 ± 0,96	5,37 ± 0,82	0	0	0
28	3,08 ± 0,97	2,69 ± 0,77	5,44 ± 0,88	0	0	0
30	3,25 ± 0,93	3,08 ± 0,50	5,21 ± 0,88	0	0	< 0,05
32	2,40 ± 0,85	2,16 ± 0,67	5,38 ± 0,63	< 0,01	< 0,05	< 0,05
33	2,56 ± 0,89	2,15 ± 0,82	5,25 ± 0,87	< 0,01	< 0,05	< 0,05
35	2,93 ± 0,64	2,93 ± 0,72	5,79 ± 0,70	< 0,5	0	0
37	2,58 ± 0,82	2,69 ± 0,55	5,67 ± 0,56	< 0,01	0	0
39	2,75 ± 0,84	2,65 ± 0,80	5,86 ± 0,51	< 0,05	0	0
43	2,71 ± 0,71	2,85 ± 0,72	5,31 ± 0,69	< 0,01	0	< 0,05
45	2,85 ± 0,80	2,32 ± 1,28	5,05 ± 0,43	< 0,05	0	< 0,001
49	2,77 ± 0,84	2,03 ± 0,79	5,11 ± 0,50	< 0,05	< 0,01	< 0,001
51	2,72 ± 0,79	2,61 ± 0,87	4,99 ± 0,45	< 0,05	0	< 0,001
55	2,64 ± 0,79	2,02 ± 1,08	4,76 ± 0,71	< 0,01	< 0,05	< 0,01
56	1,67 ± 1,01	1,12 ± 1,01	4,81 ± 0,68	< 0,001	< 0,01	< 0,01
57	2,32 ± 1,00	1,66 ± 0,92	4,50 ± 0,76	< 0,01	< 0,01	< 0,001
58	2,13 ± 0,68	1,29 ± 0,81	4,42 ± 0,68	< 0,001	< 0,001	< 0,001
59	1,67 ± 1,04	1,21 ± 1,06	4,52 ± 0,92	< 0,001	< 0,01	< 0,01
60	2,25 ± 0,96	1,59 ± 0,91	4,87 ± 0,48	< 0,01	< 0,01	< 0,001
61	2,53 ± 1,00	2,12 ± 0,65	4,66 ± 0,71	< 0,5	< 0,01	< 0,01
63	3,63 ± 0,73	2,96 ± 0,65	5,73 ± 0,54	0	0	0
C	4,00 ± 0,64	3,35 ± 0,64	6,18 ± 0,39			

LEGENDA: $\bar{x} \pm sd$ = THE MEAN log VALUE OF SALMONELLA CFU ± DEVIATION., L = LIVER., S = SPLEEN., G = GUT

Tab. 8

showed a similar protective activity of the single fractions eluted during the gel chromatography of DLE isolated from human leucocytes.

Other authors suggested differences in DLE activity dependent on the origin of DLE obtained from the peripheral blood lymphocytes (Borvák et al. 1987) or porcine spleens (Li Zailian 1987). The differences have been also detected within the spectrum of active fractions purified by means of the gel chromatography.

Using the mouse model we have estimated the highest protective activity of *Salmonella*-specific DLE in the fraction no. 58. This corresponded to (i) 10-fold concentration at $OD_{260} = 0.6$, and (ii) 10-fold concentration at $OD_{280} = 1.7$. The given extinction inhibited, in a significant degree, penetration of salmonellae into the parenchyma organs and their multiplication in the digestive tract.

Profile of the elution curve indicated, that the fractions with maximum absorbance at OD_{260} gave either low or no protective activity. On the other hand, the fractions with maximum OD_{280} have showed the highest protective activity. Characteristics of low-molecular components of DLE is given by index: $OD_{260}/OD_{280} = 1.8$. This index ranged from 0.26 to 0.34 in the fractions showing the high protective activity. In contrast, the index was in the range of 2.05–3.2 in the intact fractions. From the index of absorbance values it is possible to deduce that a marked protective activity is ensured by DLE components with molecular weight of approximately 2000–3000 daltons that are represented by oligoribonucleotides or oligoribonucleophosphopeptides responsible for the transfer of specific immunity to *Salmonella* infection. Our results are supported by data from other authors who observed the transfer of antigen-specific cell-mediated immune response both *in vitro* and *in vivo* (Dunnick and Bach 1976; Burger et al. 1979; Paddock et al. 1983; Wilson et al. 1982).

So far obtained results suggest that: (1) mouse model is suitable for testing of the protective activity of specific DLE and for the determination of the minimum inhibitory dose against salmonelosis. (2) isolated *Salmonella*-specific DLE is formed by a mixture of fractions with various activities affecting the penetration of salmonellae into the parenchymatous organs and colonization of gastrointestinal tract as well. (3) the index ratio of OD_{260} to OD_{280} characterizing low-molecular components in Sephadex fractions shows different values than in the case of crude DLE preparation.

Využitie myšieho modelu pre stanovenie protekčnej aktivity špecifického salmonelového leukocytárneho dialyzátu

Špecifický leukocytárny dialyzát (LcD^s) bol pripravený z leukocytov periférnej krvi, z mezenteriálnych lymfatických uzlín a slezín teliat, vakcinovaných a následne infikovaných virulentným kmeňom *S. typhimurium*. Nešpecifický LcD (LcD^n) bol pripravený z lymfatických uzlín výkrmových býkov. Na SPF bielych myšiach ako aj myšiach inbrednej línie C57BL/6 po aplikácii LcD a následnej infekcii kmeňom *S. typhimurium* bola testovaná inhibícia penetrácie salmonel do pečene, sleziny, ako aj kolonizácie tráviaceho traktu. U bielych myší aplikácia LcD^s navodila výraznú inhibíciu až elimináciu penetračnej schopnosti virulentného kmeňa *S. typhimurium*. U myší linie C57BL/6 LcD^s čiastočne inhiboval pomnoženie salmonel v pečeni a slezine. LcD^n neinhiboval penetráciu a kolonizáciu salmonel.

Štandardizácia LcD preparátov bola vykonaná meraním OD_{260}, LcD^s $OD_{260} = 1.5$, desaťnásobne zahustený zaistil inhibíciu až elimináciu penetračnej a kolonizačnej schopnosti *S. typhimurium*.

Frakcionácia LcD^s cez Sephadex G-25 potvrdila heterogenitu frakcií v protekcii

voči salmonelovej infekcii. Testovaný index pomerov OD_{260} k OD_{280} sephadexových frakcií sa líši od indexu LcD preparátu.

Применение мышей в качестве модели для проверки специфического салмонеллезного лейкоцитарного диализата

Специфический лейкоцитарный диализат (LcD^s) был приготовлен из мезентериальных лимфатических узлов и селезенки телят, вакцинированных и споследствии инфицированных вирулентных штаммом *S. typhimurium*. Неспецифический (LcD^n) лейкоцитарный диализат был подготовлен из мезентериалных лимфатических узлов быков. На SPF белых мышей, а также мышей имбредной линии C57BL/6 после ввода лейкоцитарного диализата и последующей инфекции штаммом S. typhimurium проверяли ингибицию проникновения салмонел в печень, селезенку, а также колонизацию пищеварительного тракта. Ввод лейкоцитарного диализата (LcD^s) у белых мышей существенно тормозит и даже исключает способность к проникновению вирулентного штамма *S. typhimurium*. У мышей линии C57BL/6 лейкоцитарный диализат лиш частично тормозит размножение вирулентного штамма Salmonella typhimurium в печени и селезенке. LcD^s неингибировал проникновение и колонизацию салмонелл в печень и селезенку.

Определение единицы активности лиофилизированного LcD, проверяемой на модели мышей, проводили измерением оптической плотности при 260 нм ($ОП_{260}$). Интраперитонеально вводимый раствор специфического $ОП_{260}$ 1, 5, сгушенный в десять раз, обеспечил выразительное торможение и даже исключение пенетрирующей и колонизационной способности вирулентного штамма S. typhimurium.

Фракционирование LcD по отношению к сальмонеллезной инфекции через сефадекс Г-25 подтвердило гетерогенность фракций в защите от сальмонеллезной инфекции.

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