Migration of Macrophages Carrying Antigen into the Thymus

Macrophages mediate the immune response to certain antigens[1]. In connection with studies on subcellular turnover and storage compartments for heat denatured bovine serum albumin (cBSA) within cells from peritoneal exudate (PEC) the question arose whether there is a functional heterogeneity among the phagocytic cells from peritoneal exudates of CBA mice.

This possibility was tested by labelling PEC in short term tissue culture with Cr^{51} and feeding them with I^{125} marked cBSA at the same time. The cells were then washed and injected i.p. into normal recipient mice and the ratio of I^{125}/Cr^{51} was determined in different organs at intervals after injection. Any alteration in the ratio of I^{125}/Cr^{51} in different organs was thought to indicate cell heterogeneity in terms of I^{125}cBSA or Cr^{51} uptake and destination of migration. The validity of this conclusion depends on 3 assumptions. (1) There is no substantial transfer of I^{125}cBSA from the phagocytic cells to other cells. It has been shown that macrophages release I^{125} as mono- and di-iodotyrosine and not as BSA[2]. (2) The Cr^{51} and I^{125} released from cells after transfer is excreted quickly and not taken up any more by other cells. This point has been studied[3]. (3) The ratio of free I^{125} and Cr^{51} in the circulation is not different from the ratio of the cell-bound label (controlled by TCA precipitation of whole blood, Table II).

The details of the I^{125}cBSA and Cr^{51} uptake by PEC in tissue culture from 4 different experiments are summarized in Table I. The distribution of radioactivity among several organs of recipient CBA mice after transfer

Table I. Uptake of I^{125}cBSA, I^{131}cBSA and Cr^{51} by PEC in tissue culture[a]

	Exp. 1, I^{125}	Exp. 2, I^{125}	Exp. 3, I^{125}	Exp. 4a, I^{131}	Exp. 4b, I^{125}
PEC/ml[b]	1×10^7	5×10^6	1.2×10^7	4.5×10^6	4×10^6
Total volume (ml)[c]	20	20	20	10	10
mg cBSA/ml[d]	0.6	0.6	4	0.03	0.03
μg uptake/mg cBSA in medium/10^8 cells	3.75	17	7	15	11
μC Cr^{51}/ml	12.5	12.5	12.5	–	–
Uptake	6×10^{-3}	3×10^{-2}	3.75×10^{-2}	–	–
μg cBSA in PEC transferred into 2 mice	30	40	220	1.25	0.9
No. of cells transferred into 2 mice	7.4×10^7	2×10^7	4×10^7	2.1×10^7	

[a] Cr^{51} as sodium chromate BP. Specific activity 300 μC/μg. I^{125} and I^{131} from the Radiochemical Centre, Amersham, in high specific activity (50–100 mC/ml). Iodide for protein iodination. Iodination according to [8]. Radioactivity was measured with a Packard autogamma spectrometer under conditions where 10% of the Cr^{51} counts went into the I^{125} channel and less than 1% of I^{125} in the Cr^{51} channel: the data were corrected for background and overlapping of Cr^{51} with I^{125}. [b] PEC from CBA mice, injected at day 3 with 3 ml thioglycolate (Difco), at day 0 washed out from the peritoneum with 4 ml tissue culture medium 199 (Glaxo). [c] Tissue culture medium 199, buffered with *Tris* pH 7.2 (0.05 mmol/ml); 4% CO_2 in atmosphere, 37 °C; 1 h incubation; Esco tissue culture dishes. [d] cBSA prepared according to [9].

Table II. Recovery of I^{125}cBSA and Cr^{51} activity in different organs from recipients of labelled PEC[a]

	90 min		2 h		4 h		9 h		20 h		4 h	
	I^{125}/Cr^{51}	Ratio	I^{125}/Cr^{51}	Ratio	I^{125}/Cr^{51}	Ratio	I^{125}/Cr^{51}	Ratio	I^{125}/Cr^{51}	Ratio	I^{125}/Cr^{51} (heated cells)	Ratio
Blood	1863 1164	1.60	1823 764	2.40	1780 1180	1.51	206 807	0.26	93 461	0.20	845 3829	0.22
Spleen	744 4269	0.18	459 1063	0.43	1252 4959	0.25	332 2953	0.11	85 3706	0.20	2440 2153	1.14
Liver	3024 7440	0.41	5550 8640	0.64	2714 6118	0.44	1687 8304	0.20	330 4266	0.08	5867 6827	0.86
Lymph nodes	–		462 855	0.54	253 675	0.38	38 209	0.18	40 471	0.09	134 327	0.41
Thymus	1047 162	6.46	890 217	4.10	825 467	1.83	146 481	0.30	13 161	0.08	42 63	0.67
PEC[b]	3447 57027	0.06	9624 42217	0.23	355[c] 5185[c]	0.07	3078 21575	0.14	< 1 6489	< 0.01	15453 13507	1.14
Supernatants of 5% TCA precipitation of blood fraction	–		486 254	1.92	584 404	1.44	48 181	0.27	15 63	0.24	256 995	0.26

[a] Counts/3 min. Organs of 2 animals pooled. [b] Cells washed before counting. [c] Approx. 75% of cells lost during washing. Data for 90 min and from 2–20 h are 2 different experiments. Input (90 min): 2.3×10^4 cpm of I^{125}/2 mice; recovery: 3.4×10^3 cpm of $I^{125} \triangleq 15\%$. 6.2×10^4 cpm of Cr^{51}/2 mice; recovery: 2.3×10^4 cpm of $Cr^{51} \triangleq 37\%$. Input (2–20 h): 6.7×10^4 cpm of I^{125}/2 mice; recovery: 2 h: 6.4×10^3 cpm of $I^{125} \triangleq 9.6\%$; 4 h: 2.4×10^3 cpm $\triangleq 3.6\%$; input: 1.2×10^5 cpm of Cr^{51}/2 mice; recovery: 1.8×10^4 cpm of $Cr^{51} \triangleq 14.5\%$; 6.3×10^3 cpm $\triangleq 5.1\%$; 4 h, heated cells: 8.2×10^3 cpm $\triangleq 12.3\%$, 8.9×10^3 cpm $\triangleq 7.2\%$.

of these cells is presented in Tables II and III. In the first 4 h after transfer the ratio of I^{125}/Cr^{51} is markedly different between the organs. It is highest in the thymus and lowest in the PEC recovered from the recipients; the difference in the I^{125}/Cr^{51} ratio between thymus and PEC is 18- to 100-fold. The difference between thymus and spleen is 5- to 35-fold. A high ratio is also found in the blood. During the first 2 h after transfer the absolute amount of I^{125}cBSA in the thymus is higher than in the spleen; at 4 h and later the opposite result is found.

It is important for the interpretation of the data that the I^{125}/Cr^{51} ratios of the supernatants of 5% TCA precipitation of the whole blood are the same as in the whole blood at any given time and that these supernatants contain only 20–30% of the radioactivity. This shows that there is no excess of one isotope in the circulation. As a further control an aliquot of the cells prepared for transfer was heated for 10 min in boiling water and then injected into mice (Table II, last column). In this group only 0.17% of the recovered I^{125} activity was found in the thymus after 4 h compared to 4.8–11.5% after injection of living cells. In addition no marked difference in the ratio of I^{125}/Cr^{51} could be found in the organs studied.

After 9 h in the recipient mice the radioactivity becomes equally distributed among the organs and the amounts recovered become low. This is partly due to a loss of cBSA-bound I^{125} by release of I^{125} mono- and diiodotyrosine. On the other hand the rate of loss of Cr^{51} from cells is 5%/h. These reasons taken together allow no meaningful calculation of the distribution for the period between 9 and 24 h after injection of the PEC.

The PEC from CBA mice induced with thioglycolate consist of about 80% macrophages as judged by histological criteria. From the amount of I^{125}cBSA carried into the thymus it was already improbable that this was transported unspecifically as a contamination of the lymphocyte population. To disprove this possibility further, PEC were incubated in tissue culture for 2 h; then cells non-adherent and adherent to the polystyrene dishes were separated. The non-adherent population contained lymphocytes and a large amount of macrophages. Adherent and non-adherent cells were then fed in tissue culture either with I^{125}cBSA (adherent) or I^{131}cBSA (non-adherent). Afterwards they were washed, pooled again, and injected i.p. into normal recipients. At intervals the distribution of I^{125}- and I^{131}cBSA in the different organs was tested. It was expected that if the radioactivity found in the thymus was brought in by lymphocytes the ratio of I^{125}/I^{131} would be lower in the thymus than in the other organs. Table IV shows that there is no significant difference in the I^{125}/I^{131} ratio between the organs tested. There can be no significant transport by non-phagocytic cells.

Table III. Recovery of I^{125}cBSA and Cr^{51} activity in different organs from recipients of labelled PEC[a] (an experiment similar to the one presented in Table II)

	4 h		18 h		24 h	
	I^{125}/Cr^{51}	Ratio	I^{125}/Cr^{51}	Ratio	I^{125}/Cr^{51}	Ratio
Blood (1 ml)	837	1.95	44	0.47	175	0.50
	430		93		349	
Spleen	844	0.95	29	0.18	147	0.32
	885		166		466	
Liver	1798	1.31	44	0.11	663	0.31
	1369		405		2100	
Lymph nodes	97	0.78	8	0.66	9	0.14
	125		12		63	
Thymus	395	5.12	< 1	< 0.01	4	0.05
	77		24		70	
PEC[b]	824	0.16	< 1	< 0.01	238	0.15
	5294		396		1563	

Injected: 2.4×10^4 cpm of I^{125}; 4.0×10^4 cpm of Cr^{51}. Recovered after 4 h: 4.8×10^3 cpm of $I^{125} \triangleq 20\%$; 8.2×10^3 cpm of $Cr^{51} \triangleq 21\%$. [a] cpm. Organs of 2 animals pooled. [b] Cells washed before counting.

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Table IV. Distribution of I^{125}cBSA and I^{131}cBSA from adherent and non-adherent PEC in organs of recipients of pooled PEC[a]

	30 min		1 h		3 h		19 h	
	I^{125}	I^{131}	I^{125}	I^{131}	I^{125}	I^{131}	I^{125}	I^{131}
Radioactivity injected into 2 mice at time 0	1.11×10^5	3.27×10^5	1.11×10^5	3.27×10^5	1.11×10^5	3.27×10^5	1.11×10^5	3.27×10^5
Radioactivity recovered from organs of 2 mice	6.7×10^4	2.0×10^5	5.0×10^4	1.6×10^5	2.7×10^4	8.6×10^4	4.8×10^3	1.2×10^4
% of injected	60	61	45	49	24	26	4.3	3.7
Ratio I^{125}/I^{131} in								
Blood (0.5 ml)		0.344		0.311		0.325		0.345
Spleen		0.305		0.364		0.387		0.357
Liver		0.328		0.310		0.342		0.358
Thymus		0.340		0.333		0.290		0.324
PEC		0.375		0.312		0.212		0.385

[a] Counts/3 min.

The high ratio of I^{125}/Cr^{51} in the thymus could mean that a population of phagocytic cells inefficient in uptake of Cr^{51} migrates preferentially into the thymus. This possibility seems unlikely since it is known that free Cr^{51} after i.p. injection into mice distributes equally into different organs[4]. Alternative explanations are that a small population of PEC takes up I^{125}cBSA quickly, or stores it efficiently, or that the thymus provides macrophages with a favourable environment for retaining antigen.

It is known from studies using S^{35}-BSA that antigen can enter and is retained in the thymus[5]. Macrophages have also been observed in the thymus[6], where in part they may act as scavangers; but they have also been found in close contact with lymphocytes in mitosis within the thymus of AKR mice[7]. It is concluded from the experiments reported in this communication that efficient presentation of antigen to thymus lymphocytes by other cells may be a part of the normal immune response[10].

Zusammenfassung. Makrophagen aus dem Peritoneum von CBA-Mäusen, die in Zellkultur hitzedenaturiertes Rinder-Serumalbumin phagozytiert haben, transportieren dieses bis zu 4 h nach Transplantation vornehmlich in den Thymus normaler Empfängertiere.

E. Kölsch[11]

National Institute for Medical Research, Mill Hill, London, N.W.7 (England), 15 March 1968.

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[11] Present address: Institut für Genetik der Universität, 5 Köln-Lindenthal (Germany).

Transvascular Passage of Albumin-I^{131} into Skin of Immunized Mice

It is possible that changes in the connective tissue-interstitial space may affect the manner and the rate of transport of molecules to and from the circulatory system and the cells. It has been proposed that this sequence may play a significant role in aging and that the connective tissue changes may result from genetically programmed chronogenic phenomena and exogenously derived pathogenic alterations[1]. Among the latter are immunologic processes. Antigen-antibody interactions are known to occur in the interstitial space and in basement membranes[2]. These interactions and their sequalae might result in alterations in the diffusibility characteristics of the milieu through which molecules must pass on their way to and from the cells. This study was undertaken in order to test this hypothesis.

Method. Male mice weighing 28 ± 1 g were obtained from a commercial supplier and maintained on Purina pellets. 1 group of animals was injected s.c. with 0.5 ml of a 5% solution of sodium caseinate, 5 times a week for a period of 6 weeks according to the procedure of Christensen and Hjort[3]. Each animal received a total of 750 mg of sodium caseinate. Another group received a s.c. injection of 0.2 ml streptococcus group A extract (BBL-74027B) in complete Freund's adjuvant and 0.1 ml of the soluble antigen i.p. 4 weeks later.

48 h following the last injection of sodium caseinate, and 2 weeks after the last injection of streptococcal antigen, these animals and their controls were injected in a tail vein with 1 μc of human albumin-I^{131} in a volume of 0.1 ml. In some animals, after 4 min and in others after 2 h, samples of blood were obtained from the internal occular plexus and the mice were killed by cervical fracture. The skin was removed in a uniform manner, dehaired, scraped free of s.c. tissue, dissolved in formic acid, and assayed for bound I^{131}, as previously described[4]. 0.02 ml of serum was used for I^{131} assay.

Results and discussion. The results are shown in the Table. As in the previous experiments[4], the 4 min values were assumed to correspond to the mixing time in the plasma and the radioactivity in the skin was thought to be due to the circulating plasma.

The mean plasma volumes in the skin calculated from 4 min values were 0.0309 ml, 0.0411 ml and 0.0435 ml, respectively, in the control, casein- and strep-antigen treated animals. After 2 h, the serum radioactivity decreased to 64.2%, 77.9% and 91.5%, respectively, of the

Albumin-I^{131} in serum and skin of immunized mice

	No.	Weight (g)	Serum (cpm 0.02 ml)	Skin (cpm total)
4 min				
Control	8	39.2	11990 ± 947[a]	18561 ± 2061
Casein	8	29.8	10026 ± 793	20623 ± 1037
Strep-antigen	7	34.3	8537 ± 1039	18589 ± 1872
2 h				
Control	8	39.5	7695 ± 514	27173 ± 1678
Casein	8	29.1	7810 ± 520	30027 ± 1101
Strep-antigen	7	34.7	7808 ± 320	29023 ± 2451

[a] Standard error of the mean.

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