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J.W. Quin, Department of Experimental Pathology, John Curtin School of Medical Research, The Australian National University, P.O. Box 334, Canberra City, A.C.T. 2601, Australia

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Studies on the Immunochemical Composition of Human Thoracic Duct Lymph of Patients with Rheumatoid Arthritis and Polymyositis

Seymour Levine, David Tak Yan Yu, Sheng Hsu, Herbert Machleder, Harold E. Paulus, Eugene V. Barnett

Departments of Medicine and Surgery, UCLA Center for the Health Sciences, Los Angeles, California, U.S.A.

Summary

Analysis of immunoglobulins, autoantibodies and third component of complement was carried out on the thoracic duct lymph of 6 patients with rheumatoid arthritis and 2 patients with polymyositis. It was found that the distribution of these immunoproteins between lymph and serum reflected the distribution of total protein and did not appear to be dependent on molecular size. There was no significant difference in the distribution of the macromolecule IgM as compared to the other immunoglobulins. IgD was an exception with an unanticipated and unexplained lower concentration in lymph and higher serum to lymph ratio when compared to the other immunoglobulins.

Introduction

Lymphocytes are thought to play an important role in the pathogenesis of autoimmune disease. We have studied the effects of lymphocyte depletion by thoracic duct drainage (TDD) in patients with rheumatoid arthritis and have found that such patients undergo significant clinical improvement during prolonged continuous removal of thoracic duct lymphocytes through a surgical fistula (1). Immunoproteins such as immunoglobulins, autoantibodies and complement are also thought to play a role in the pathogenesis of autoimmune diseases.

Some studies of immunoprotein levels in the central lymph of humans have been previously published although nearly all in patients with non-rheumatic disease. *Dumont* and co-workers (2) showed that initial gamma globulin levels in lymph were about one half that in serum and declined from five to ten fold in 10 patients who underwent lymph depletion by TDD for up to 8 days with no reinfusion of lymph. Total protein and gamma globulin levels declined in both serum and lymph in these patients, with serum gamma globulin levels falling to less than one

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half the levels in control samples. In one patient with rheumatoid arthritis, rheumatoid factor levels were the same in lymph and blood.

Tunner and his colleagues (3) showed that lymph immune globulins were about one half the serum level in 9 patients who underwent lymph depletion by TDD for an average of 4.5 days. The lymph was not reinfused. Total protein and immunoglobulin concentration in the serum all declined with lymph depletion.

Tilney and Murray (4) in 14 renal transplant patients undergoing TDD for 4 to 42 days as an adjunct to immunosuppression, found lymph proteins to be between one half to two thirds those of serum levels. Despite reinfusion of cell-free lymph there was a modest decline in serum proteins, and all protein fractions in lymph, except the alpha globulin, decreased to about 60% of the initial concentrations during and shortly following the drainage period.

Graber and co-workers (5) in 2 patients with chronic renal failure undergoing lymph dialysis showed that beta one C globulin and total complement levels in lymph were on third that of serum. Levels of immunoglobulins A and M in the lymph were one third to one half the level in serum while the level of immunoglobulin G was one tenth that of serum.

Workers at the University of Texas Medical Branch, Galveston (6-9), demonstrated that when cell-free lymph was returned to the patient, total protein and immunoglobulin levels in serum and lymph changed little and were maintained at satisfactory levels despite profound total body depletion of lymphocytes in uremic patients undergoing TDD as an adjunct to immunosuppression. In the present study we have carried out immunoprotein analyses on the thoracid duct lymph of 8 patients with rheumatic disease and compared these results to similar analysis of the serum of the same patients.

Methods

Eight caucasian females undergoing lymphocyte depletion by prolonged TDD were studied; six had classic rheumatoid arthritis (RA) and two had polymyositis (PM) (Table 1). Their ages ranged from 25-62 years at the time of thoracic duct cannulation. The duration of lymph drainage is shown in Table 1. Methods for collection and preparation of cell-free lymph have been previously reported (10). Prior to analysis, fat was removed by centrifugation at 25,000 g for 30 minutes. During continuous TDD, sterile cell-free lymph was reinfused intravenously in order to avoid protein depletion. Blood was drawn from an antecubital vein, and serum was removed for analysis and chemical comparison to cell-free, fat free lymph. Lymph specimens were obtained by removing an aliquot of a standard, well mixed, pooled 24 hour lymph collection. Whenever possible, specimens were obtained on the same day although this was not always the case early in TDD. No patient received immunosuppressive therapy during the two months prior to TDD.

Table 1. Patient population

Patient	Diagnosis	Age	Duration of TDD (days)
IP	RA	25	45
MB	RA	47	20
JC	RA	49	7
CR	RA	51	4
JR	RA	54	80
DM	RA	49	26
TM	PM	62	11
PM	PM	37	80

RA = Rheumatoid Arthritis
PM = Polymyositis
TDD = Thoracic Duct Drainage

Concentrations of immunoglobulin classes G, A, M, and D (IgG, IgA, IgM, and IgD) were determined using *Hyland* immunodiffusion plates (11). Antinuclear antibody titers (ANA) were determined by immunofluorescence using human leukocytes as the substrate (12). Latex fixation tests were performed by the *Hyland* latex microtiter method (13). Sensitized sheep cell agglutina-

tion titers (SSCA) and heterophile antibody titers were determined by standard methods (14). Levels of the third component of complement (β-1-C) were determined by the *Hyland* immunoplate technique (15). DNA-binding was determined by a modification of the *Farr* test (16).

Results

The results are shown in Tables 2-4. Table 2 shows the concentration of total protein, immunoglobulins G, M, A, and D and the third component of complement (β-1-C) in serum and thoracic duct lymph. The ratios of serum to lymph concentrations are listed for each protein and for total protein. Because the concentration of each protein in the lymph depends to some extent on the lymph flow rate, the serum to lymph ratios of these immunoproteins are divided by the serum to lymph ratio of total protein concentration for each pair of specimens in order to correct for the protein dilution that occurs when lymph flow rates are high. In general, the concentrations of each immunoglobulin class and β-1-C were higher in serum than in lymph. There were no significant differences in the *mean* ratio of serum to lymph (S/L) to total protein concentration of serum to lymph (S_{TP}/L_{TP}) among immunoglobulins G, M, and A and β-1-C; nor was there any consistent trend in individual ratios as TDD progressed in those patients who had more than one sample analyzed (IP, MB, JR and PM (Table 3). Indeed, the wide scatter of these ratios among individual patients appeared to be random. The clustering of the mean ratios for IgG, IgM, IgA and β-1-C around 1.00 suggests that the distribution of these proteins reflects the distribution of total protein. IgD was an exception with a much lower concentration in lymph and a much higher mean ratio (Table 3) than anticipated (Table 2).

Due to the small number of observations available for each patient, no definite conclusions can be drawn from these data as to the effect of progressive TDD on the S/L: S_{TP}/L_{TP} ratios for each of the above components. Table 2 shows no consistent increase or decrease in the absolute levels of immunoglobulins or β-1-C as TDD progressed in time.

Table 4 shows the reciprocal of titers for latex fixation, SSCA, heterophile and ANA as well as the percent of DNA binding levels in serum and lymph. In general, titers were higher in the serum specimens although some exceptions are noted. In those patients who had more than one sample studied and who had positive titers ($>$ 1:80) on baseline examination (IP, MB and JR), the latex fixation test showed a decrease in titer of 2 to 4 tube dilutions in the serum and 0 to 7 tube dilutions in the lymph as TDD progressed. There were more modest decreases in the SSCA titers in these patients as well. In patient IP, there was a four fold decrease in ANA titer in serum and lymph as TDD progressed. In patient JR there was a similar four fold decrease in serum titer and a two fold decrease in lymph. No clear trend was present in the DNA-binding although there was an apparent decrease in levels during the course of TDD.

Discussion

Previous studies have revealed that the chemical composition of human thoracic duct lymph reflects that of blood serum, but with reduced levels of the various chemical constituents in the lymph (2-5, 17-19). In this study we observed that the immunochemical composition of human thoracic duct lymph follows these same general principles. Several groups (2, 3, 4, 18) have reported that the protein concentration in human thoracic duct lymph is about 1/2 to 2/3 that of blood serum, while the corresponding value for the macroglobulin fraction was much lower – about 15% (18). *Bergstrom and Werner* (18) interpreted these data as suggesting that the macromolecules penetrate the blood-lymph barrier less readily than other protein molecules. *Yoffey and Courtice* (20) support this thesis that molecular size is the most important factor in determining how readily a plasma protein will move through the blood capillary wall, enter the interstitial fluid and end up in the lymph.

Table 2. Total protein concentration (TP) (gm/100 ml), immunoglobulin concentration (mg/100 ml) and third component of complement concentration (β-1-c) (mg/100 ml) in serum (S) and thoracic duct lymph (L) with S/L ratio for each protein

Patient	Specimen	No. days TDD*	Total Protein Conc.	Total Protein S/L	IgG Conc.	IgG S/L	IgM Conc.	IgM S/L	IgA Conc.	IgA S/L	IgD Conc.	IgD S/L	β-1-C Conc.	β-1-C S/L
IP	S	2	5.7	1.4	570	1.1	59	2.1	170	2.1	7.2	9	125	2.2
	L	4	4.0		530		28		83		0.8		57	
	S	20	5.2	1.2	355	1.4	75	1.4	135	2.9	15.5	17.2	110	2.0
	L	20	4.5		244		59		65		0.9		56	
MB	S	0	7.2	2.5	1240	3.0	175	2.7	135	2.2	17.5	3.9	105	2.6
	L	1	2.9		417		65		61		4.5		40	
	S	20	4.8	2.2	190	0.4	52	1.2	55	0.8	19.5	17.7	56	1.7
	L	18	2.2		436		44		73		1.1		33	
JC	S	−1	7.3	1.8	1225	1.6	311	2.0	275	1.3	17.5	0.9	155	2.3
	L	1	4.1		763		155		208		20.3		78	
CR	S	−22	5.7	2.2	610	1.3	250	1.5	215	1.4	9.2	6.6	155	1.6
	L	1	2.6		533		167		155		1.4		98	
JR	S	3	7.8	2.1	1150	1.3	195	1.9	170	1.7	3.7	0.2	155	1.4
	L	0	3.8		910		101		103		17.2		110	
	S	20	6.1	1.8	1150	2.1	66	1.6	155	2.2	14.0	14.0	130	2.3
	L	20	3.6		540		41		68		1.0		57	
	S	76	6.6	1.7	1335	1.3	135	3.0	170	2.8	12.5	8.3	150	2.7
	L	76	3.8		994		43		60		1.5		55	
DM	S	−2	7.8	2.0	1450	2.7	95	2.6	235	2.4	5.2	5.8	100	1.7
	L	1	3.9		542		36		98		0.9		59	
TM	S	−1	6.3	1.8	720	2.5	155	2.6	300	3.4	9.2	7.7	210	4.3
	L	2	3.5		302		58		88		1.2		49	
PM	S	0	6.9	2.7	980	3.5	85	1.9	185	3.7	11.0	6.9	170	2.0
	L	2	2.6		276		45		56		1.6		85	
	S	18	6.8	1.7	1005	1.5	155	2.3	255	1.9	3.7	1.4	140	1.3
	L	18	4.1		650		68		138		2.7		105	
	S	47	6.8	2.4	1225	2.5	95	1.2	200	2.3	4.6	2.1	165	2.3
	L	47	2.8		480		82		86		2.2		72	

* = number of days after thoracic duct drainage was begun when specimen was obtained
– = indicates specimen was obtained prior to cannulation of thoracic duct
Conc = concentration
TDD = Thoracic duct drainage

Table 3. Ratio of serum to lymph concentration (S/L) for immunoglobulins and 3rd component of complement (β-1-C) to serum to lymph concentration of total protein (S_{TP}/L_{TP}).

Patient	No. days TDD*	S/L: S_{TP}/L_{TP}				
		IgG	IgM	IgA	IgD	β-1-C
IP	2	0.79	1.50	1.50	6.42	1.57
	4					
	20	1.17	1.17	2.42	14.3	1.67
	20					
MB	0	1.20	1.08	0.88	1.56	1.04
	1					
	20	0.18	0.55	0.36	8.05	0.77
	18					
JC	−1	0.89	1.11	0.72	0.50	1.28
	1					
CR	−22	0.59	0.68	0.64	3.00	0.73
	1					
JR	3	0.62	0.90	0.81	0.10	0.67
	0					
	20	1.17	0.89	1.22	7.78	1.28
	20					
	76	0.76	1.76	1.65	4.88	1.59
	76					
DM	−2	1.35	1.30	1.20	2.90	0.85
	1					
TM	−1	1.39	1.44	1.89	4.28	2.39
	2					
PM	0	1.30	0.70	1.37	2.56	0.74
	2					
	18	0.88	1.35	1.12	0.82	0.76
	18					
	47	1.04	0.50	0.96	0.88	0.96
	47					
		IgG	IgM	IgA	IgD	β-1-C
Mean		(n=14)	(n=14)	(n=14)	(n=14)	(n=14)
S/L : S_{TP}/L_{TP}		0.95	1.07	1.20	4.15	1.16
SD		0.34	0.38	0.54	3.93	0.50
$S\bar{X}$		0.09	0.10	0.15	1.05	0.13

SD = Standard deviation.
$S\bar{X}$ = Standard error of mean.
* = Number of days after thoracic duct drainage (TDD) was begun when specimens were obtained.
– = Minus sign indicates specimen obtained prior to cannulation of thoracic duct.

For immunoglobulins (Table 2), the concentrations in lymph ranged from 1/3 to 2/3 that of blood as has been previously reported (3, 5). Most values were about one-half that of blood, except for IgD. No significant differences were apparent when the serum to lymph ratios for IgM were compared to the lower molecular weight immunoglobulins G and A nor were there

Table 4. Latex fixation, sensitized sheep cell agglutination (SSCA), heterophile antibodies (Het), and antinuclear antibody (ANA) titers and percent of DNA binding (DNA%) levels in serum (S) and thoracic duct lymph (L). (Titers of antibodies are expressed as reciprocal.)

Patient	Specimen	No. days after cannulation	Latex	SSCA	HET.	ANA	DNA%
IP	S	2	2560	2048	8	64	18
	L	4	320	256	4	4	14
	S	20	640	1024	4	16	17
	L	20	320	256	2	Undil.	10
MB	S	0	10240	2048	Undil.	Undil.	12
	L	1	320	256	Undil.	Undil.	10
	S	20	640	256	4	Undil.	7
	L	18	20	4	2	4	NPS
JC	S	−1*	20480	2048	4	4	18
	L	1	5120	4096	Undil.	Equiv.	20
CR	S	−22	10240	2048	2	Neg.	24
	L	1	20480	1024	Undil.	4	30
JR	S	3	5120	512	2	64	21
	L	0	40960	2048	Undil.	8	24
	S	20	5120	2048	2	16	21
	L	20	1280	64	2	4	NPS
	S	76	1280	64	4	16	19
	L	76	320	8	2	4	NPS
DM	S	−2	1280	128	8	Neg.	15
	L	1	160	8	2	Undil.	9
TM	S	−1	160	4	2	Neg.	12
	L	2	40	2	2	Undil.	NPS
PM	S	0	20	4	2	Undil.	16
	L	2	640	4	4	Equiv.	NPS
	S	18	20	8	8	Undil.	21
	L	18	20	4	Undil.	Undil.	NPS
	S	47	20	4	4	–	
	L	47	20	2	2	–	

Undil. = positive in undiluted tube only.
* = minus sign indicates specimen obtained prior to thoracic duct cannulation.
Neg. = negative.
NPS = no precipitated sediment.

any significant differences in the mean ratios of S/L:STP/LTP among immunoglobulins G, A and M (Table 3). Possible explanations include: a) an active mechanism to transport IgM molecules from blood to lymph in patients with rheumatic diseases; b) existence in these patients of pathologically altered capillaries that leak IgM and other large molecules into the interstitial fluid and thence into the lymph; c) IgM synthesis in lymph nodes with resultant direct access to lymph and, d) finally, these results may be completely fortuitous.

In addition, although the absolute values for immunoglobulins varied within rather wide ranges, and there was a wide scatter of S/L:STP/LTP ratios for immunoglobulins among individual patients, the mean ratios (Table 3) clustered about 1.00 for IgG, IgM and IgA suggesting that the distribution of these proteins reflects the distribution of total protein. The low lymph concen-

trations and high S/L:STP/LTP ratios for IgD were unanticipated and are unexplained and require further study.

Analysis of immunoglobulin levels in four patients who had more than one sample analyzed revealed no consistent trend in either absolute values or in S/L:STP/LTP ratios as TDD progressed. This confirms previous reports (6-9) in which patients underwent continuous reinfusion of their cell free lymph.

Several auto-antibodies thought to be of importance in the pathogenetic mechanisms of rheumatic disease are found in lymph (Table 4), although in lower titers than in blood, again reflecting the blood-lymph protein barrier alluded to above. Analysis in three patients reveals a significant lowering of titers in serum and lymph as TDD progressed, suggesting that long term lymphocyte depletion may remove cells that are involved in the production of these antibodies.

β-1-C, representing the 3rd component of the complement series, is found in both serum and lymph (Table 2) with the lymph concentration about half that of the serum, about as expected for proteins of this molecular weight. This confirms previous data (5). The fact that there was no significant decline in β-1-C level with progressive TDD may reflect the fact that these patients had their cell-free lymph reinfused, repleting this protein, or that they had an increase in synthesis of β-1-C because of their rheumatic disease, or both.

We conclude that the distribution between lymph and serum of immunoglobulins, autoantibodies and third component of complement in patients with chronic inflammation due to RA or PM reflects the distribution of total protein and does not appear to be dependent on molecular size. There was no significant difference in the distribution of the macromolecule IgM as compared to the other immunoglobulins. IgD is an exception in that the high S/L:STP/LTP ratio and low concentration in lymph for this immunoglobulin were not anticipated and remain unexplained.

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Harold E. Paulus, M.D., 1000 Veteran Avenue, Los, Angeles, California, U.S.A.

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Selective Staining of Fibrous Connective Tissue Capsules and Lymphatics

An Evaluation of "Interstitial" Fluids

Simon Rodbard

Department of Cardiology, City of Hope Medical Center, Duarte, California, U.S.A.

Summary

Ringer's solution containing ferrocyanide ion was infused into the arterial system of limbs. Some of these ions filtered across the blood capillary wall into the adjacent extracapillary fluids, from which diffusion caused these ions to enter the wall of the enclosing epimysial capsule. *Ringer's* solution containing ferric ion was then injected into the parenchyma (not intravascularly). In muscles, the intensely blue ferric ferrocyanide (Prussian blue) precipitate appeared on the walls of the fibrous connective tissue capsules that enclosed each small cluster of muscle cells, and in the lymphatics of the extracapsular clefts. No Prussian blue appeared inside the capsules. These results indicate that "interstitial" fluids are divided into two discrete pools: (a) an intracapsular pool of capillary ultrafiltrates, and (b) an extracapsular pool in the trabecular clefts. Certain implications concerning the mixing of the tissue fluids, the estimate of capillary filtration rates and some of the functional differences between intravascular and intramuscular injections are discussed.

Collagenous fibers and their associated ground substances are nearly ubiquitous in animal tissues. Much of the collagen is arranged in sheets that enclose specific structures. For example, each muscle fiber is enclosed in a delicate endomysial sleeve of collagen. A larger fibrous connective tissue capsule (epimysium) encloses each cluster of approximately 100 muscle fibers (1). Similar connective tissue encapsulations organize glands, bundles of nerve fibers (2) and other tissues into modules (3). Larger and stronger fascial enclosures hold clusters of capsules together to form tissue compartments (4).

Three-dimensional fibrous sheaths appear as lines in tissue sections and their roles as enclosures are seldom noted. Despite their omnipresence, attention is usually focused on the identification