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Immune Proteins and Other Biochemical Constituents of Peripheral Lymph in Patients with Malignancy and Postirradiation Lymphedema

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Summary

Concentration of immunoglobulins and complement proteins were studied in a group of 33 patients with localized tumors and lymphoproliferative disorders. Generally, low levels have been found, in many cases below the lowest limit of the control group. The reductions in concentration were more pronounced in patients with lympho-proliferative disorders than with solid tumors. The most reduced were IgM, C1g and total complement hemolytic activity. In a group of 8 patients with lymphedema of lower extremity complicating therapy for uterine cancer an increase of IgM and IgA and decrease in hemolytic activity were found. This indicates to the existence of a chronic inflammatory process, typical for tissues deprived lymphatic outflow.

In neoplastic diseases impairment of cellular and humoral immune response are observed (1, 2, 3, 4, 5), dependent on the stage and type of the disease, and also influenced by chemotherapy, radiotherapy and superimposed infections. The level of immune proteins in serum may be decreased, often selectively of individual classes of immunoglobulins or complement components. Also pathological monoclonal proteins may occur. Low level of immune proteins in serum may affect their concentration in tissues. Observations indicate that immune defence in the interstitial fluid and lymph might be weaker than in blood. The reason for this may be a low concentration of immune proteins in the interstitial fluid and lymph, caused by their physiologically restricted extravasation. Low level of immune proteins in serum in malignancies will cause an additional reduction of their concentration in tissues. This may be of clinical significance. It has been shown in animal experimentation that antitesticular antibody does not enter the testis in sufficient amounts unless there is an increased permeability of capillaries (6). Cytotoxic antisera active *in vitro* may have an opposite effect *in vivo*, probably due to restricted extravasation to the tissues (7). High levels of immune proteins in the tissue fluid are necessary for the destruction of some types of tumour cells located in the tissues (8, 9). Furthermore, several antineoplastic and antibacterial drugs bind to albumin (10). Their tissue concentration of serum albumin, often reduced in neoplastic diseases, and transport of that protein to the tissue fluid.

Lymphedema of lower extremity complicating the surgical and radiation therapy of uterine cancer is not an uncommon entity (11). It is a highly disabling condition restricting mobility of limb, complicated by bursting pains and recurrent lymphangitis. The skin of the lymphedematous limb deprived of lymphatic drainage, behaves like an immunologically privileged site, plagued by infections. Lymph stasis in human limbs is accompanied by recurrent lymphangitis in about 50% of cases (12).

The purpose of the present study was to measure the concentration of immune proteins and other biochemical constituents of peripheral lymph in patients with solid tumors, lymphoproliferative disorders and lymphedema developing after surgery and radiotherapy for treatment of cancer of uterus. It should be considered as a preliminary study performed in a heterologous group of patients in which we tried to detect the tissue fluid and lymph changes of clinical importance.

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Material and Methods

Fourty-one patients divided into 3 groups were studied. Group 1 consisted of 21 male patients with solid tumors as seminoma (8 patients), cancer of testis, penis or bladder (10 patients), teratomas (3 patients). Group 2 comprised 12 male patients with lymphoproliferative disorders as Hodgkin's disease of all stages (4 patients), non-Hodgkin's lymphoma of all stages (7 patients) and chronic lymphatic leukemia (1 patient). Group 3 consisted of 8 female patients with carcinoma of uterus and lymphedema of the lower limb. The age of patients ranged between 22 and 74 years. Ten patients of group 1 and 2 had chemotherapy or irradiation prior to the study and all patients of group 3 undervent hysterectomy and radiotherapy 1–5 years ago. No clinically detectable cardiac decompensation and vascular changes in lower limbs were noted. In group 3 profuse swelling of the left lower limb was found in 3 patients and of the right in 2. The duration of swelling ranged between 3 and 8 months. On lymphangiography complete blockage of lymphatics was found in the pelvis, with the dermal backflow of the contrast medium.

Lymph collection

Lymph was collected from leg superficial lymph vessel in males, and from dorsum of the foot in females. The technique of lymphatic cannulation has been described previously (13). The patients were allowed to walk and carry out their normal daily activities. No treatment was given during the period of lymph collection. The details of collection of samples of lymph and blood have been described elsewhere in this volume (see page 156).

Biochemical constituents

Total protein, immunoglobulins, complement proteins, complement hemolytic activity, enzymes, electrolytes and other constituents were measured using methods described elsewhere in this volume (see page 157).

Statistical evaluation

Data obtained in group 1 and 2 have been presented as individual values of each patient and compared with the normal values obtained by us in a group of healthy volunteers (14). Data in group 3 have been presented as means ± 1 S.E. and compared with the values obtained in a group of primary lymph stasis published previously (12).

Results

Group 1: Results have been presented on Fig. 1 and 2. The levels of serum total protein and globulin in patients with solid tumors remained within normal limits. In some cases reduced lymph concentrations was found. Several patients revealed hypo-IgG-nemia and low lymph IgG. The serum IgA was in some cases above normal but IgM remained within normal limits.

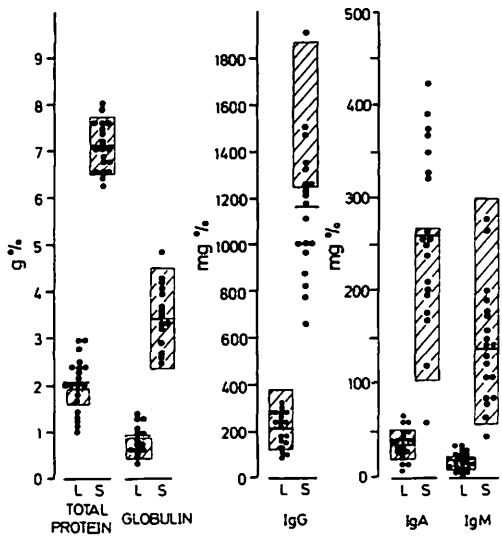

Fig. 1 Leg lymph and serum concentrations of total protein, globulins and immunoglobulins in 21 patients with localized tumors. Dots represent means of 2–6 measurement in one patient, horizontal lines means of all patients, hatched areas means ± 2 S.D. of the control group consisting of healthy individuals aged 19–27.

The lymph IgA and IgM levels were extremely low in 3 and 4 patients, respectively. Total complement hemolytic activity in serum was in 9 patients below normal, the same was found in lymph. The values of all serum complement components were largely scattered, with most within normal limits. In some cases there was low C1q and high C4. In lymph only

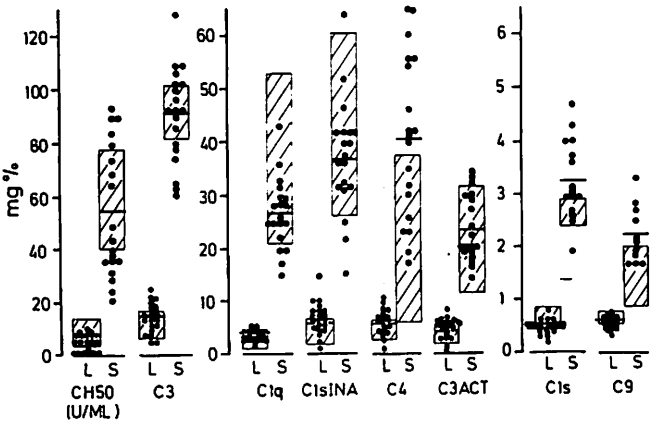

Fig. 2 Leg lymph and serum complement hemolytic activity and complement protein concentration in 21 patients with localized tumors. For details see Fig. 1.

a few patients had the level of components below the lowest normal range.

Group 2. Total protein and globulin concentration in serum remained almost within normal limits, the lymph concentrations were in some patients considerably decreased (Fig. 3). Low IgG concentration was found in serum of a majority of cases, and also a markedly reduced level in lymph. There were several cases of profound hypo-IgA- and IgM-nemia. In these patients the two classes of immunoglobulins in lymph were almost undetectable. Several patients had serum levels of complement components below the normals (Fig. 4). In lymph the levels of components were in most cases extremely low, with the C1q practically undetectable.

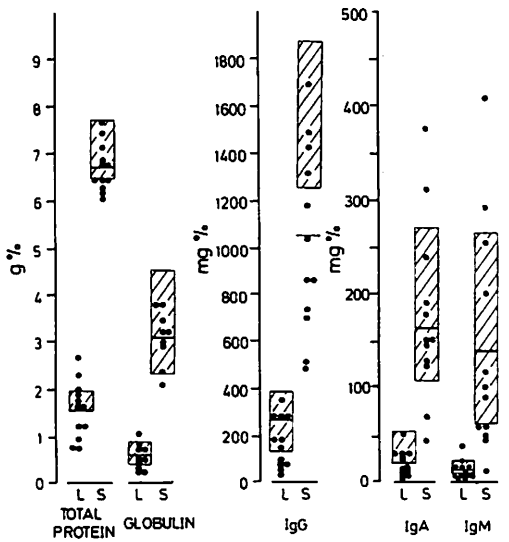

Fig. 3 Leg lymph and serum concentrations of total protein, globulins and immunoglobulins in 12 patients with lymphoproliferative disorders. For details see Fig. 1.

Group 3. Total protein concentration in lymph in cases with obstructive lymphedema was high, there was also a relatively high level of γ-globulins, IgM and IgA (Table 1). The lymph C3 concentration was high, but L/S ratio remained normal what might be attributed to the elevated serum C3. The total complement hemolytic activity was very low. Enzyme L/S ratios were lower than in the control group, with the alkaline phosphatase significantly reduced (Table 2). The level of other constituents including electrolytes was unaltered (Table 3 and 4).

Discussion

The main finding of the present study has been low levels of immunoglobulins and complement component proteins in peripheral lymph of patients with malignant diseases. These levels were in many cases below the lowest limit found in the control group of normal healthy men. The reductions in concentration were more expressed in patients with lympho-prolifefative disorders than with localized tumors. Low levels of immune proteins in lymph followed low serum levels, but there were also cases with reduced lymph and normal serum concentrations. The low total hemolytic com-

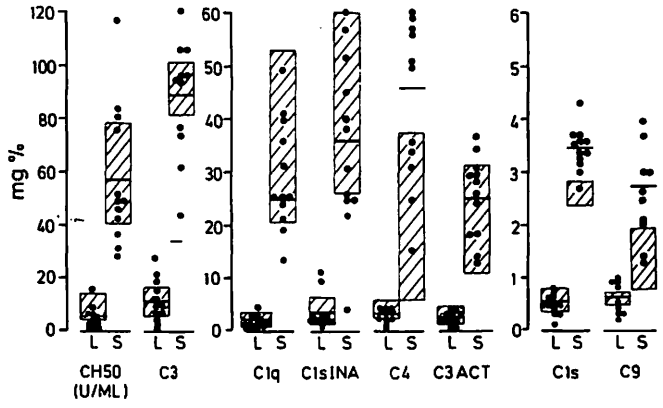

Fig. 4 Leg lymph and serum complement hemolytic activity and complement protein concentration in 12 patients with lymphoproliferative disorders. For details see Fig. 1.

plement in both groups of patients seems to be of clinical importance. It followed in some cases low complement titre in serum. The reason for it might be low level of complement components in lymph, as well as some degree of systemic activation.

Lymph concentration of proteins depends on factors influencing transport through the capillary wall and on the concentration within the vascular compartment. Increased venous pressures found in congestive heart disease and peripheral venous disorders may increase lymph flow with subsequent fall in protein concentration. No such disorders were detected in our patients.

The protein concentration in serum may directly affect lymph concentration. Variations of serum immunoglobulins in normal subjects over long periods of time are small, not exceeding 15% (15). The environmental challenge makes the major contribution to immunoglobulin variation. There is a continuous increase of IgG and IgA. The IgM levels are highest during the fifth decade. This is in contrast with the distinct decrease in the titre of antibodies against specific bacterial and viral antigens in the elderly (15). No data in the literature concerning age variations of complement protein level have been found.

In our study the values of serum immunoglobulin and complement protein concentration from patients of different age, ranging between 22 and 74 were compared with the data from a control group consisting of normal males aged 19–27. No control group of older age was available. This may make interpretation of some results difficult. However, in our group the values of serum immunoglobulins were rather low, whereas, with age there is a tendency toward increased serum levels (15).

Our findings of markedly reduced levels of immune proteins in peripheral lymph, most evident in patients with lymphoproliferative disorders, indicate that the local humoral defence may be weakened. The other important observation was that the normal serum level of proteins does not necessarily indicate a normal tissue concentration. Factors responsible for the inability of maintaining proper tissue concentration of immune proteins are not clear. Both changes in capillary transport and reduction in serum levels seem to be responsible. Several of our patients had a increased lymph flow. High lymph flow is physiologically accompanied by low protein concentration. This higher flow might have been caused by some effects of previous chemo- or radiotherapy, but this would require special studies.

The purpose of our studies in patients treated for cancer of uterus complicated by lymphedema was to show the changes in proteins, enzymes, and other constituents for detection of humoral immune defects and metabolic disturbances, usually accompanying interruption of protein recirculation via blood-tissue-lymph. The relatively high lymph protein and globulin level which we have observed, as compared with normal subjects and patients with the so-

Tab. 1 Protein immunoglobulin and C3 protein concentration and total complement activity in leg lymph of 3 patients with obstructive (postsurgical and postirradiation) and 3 patients with primary lymphedema. Mean values of 3 measurements in each patient ± 1 S.E.

		Type of Lymphedema			
		Obstructive	L/S	Primary	L/S
Total protein (g%)		3.69 ± 0.28	0.53	1.97 ± 0.11	0.24
Albumin (%)		62.5	1.1	63	1.1
Globulin (%)	alpha 1	4	0.8	4.7	0.94
	alpha 2	8.5	0.94	11.8	1.3
	beta	9.7	0.8	12.2	1.0
	gamma	14.7	0.91	9.7	0.6
IgG mg%		182 ± 43	0.28	102 ± 28	0.16
IgA mg%		118 ± 23	0.42	90 ± 11	0.32
IgM mg%		58 ± 12	0.42	18 ± 2.4	0.12
C2 mg%		49 ± 10	0.24	34.7 ± 3	0.17
CH50 μ/ml		2	0.05	2	0.07

Tab. 2 Enzyme activity in leg lymph of 5 patients with obstructive (postsurgical and postirradiation) lymphedema and 5 patients with primary lymph stasis. Mean values of at least 3 measurements in each patient.

	Type of Lymphedema			
	Obstructive		Primary	
	units/ml	L/S	units/ml	L/S
SGOT	7.69 ± 1.15	0.28 ± 0.03	4.8 ± 1.1	0.47 ± 0.1
SGPT	5.7 ± 0.9	0.20 ± 0.05	6.8 ± 1.2	0.38 ± 0.06
Acid phosphatase	4.8 ± 2.15	0.24 ± 0.06	1.23 ± 0.45	0.49 ± 0.08
Alkaline phosphatase	6.8 ± 2.5	0.13 ± 0.02	1.66 ± 0.8	0.65 ± 0.09
LDH	69.2 ± 10.2	0.58 ± 0.08	48.9 ± 10.4	0.55 ± 0.13

Tab. 3 Concentration of lymph biochemical constituents in legs of 5 patients with obstructive (postsurgical and postirradiation) and 5 patients with primary lymphedema. Mean values of at least 3 measurements in each patient ± 1 S.E.

	Type of Lymphedema			
	Obstructive		Primary	
	mg %	L/S	mg %	L/S
Urea	19.2 ± 2	1.0 ± 0.05	18.9 ± 5.3	0.9 ± 0.12
Creatinine	1.41 ± 0.08	1.08 ± 0.08	1.35 ± 0.1	0.9 ± 0.15
Creatine	0.84 ± 0.06	0.84 ± 0.06	0.9 ± 0.15	1.0 ± 0.1
Glucose	98.4 ± 15.4	1.16 ± 0.12	108.6 ± 12.0	1.3 ± 0.07
Total cholesterol	23.0 ± 1.0	0.11 ± 0.01	32.6 ± 0.01	0.18 ± 0.03

called primary non-obstructive lymphedema, has most likely been caused by chronic inflammation and accumulation of proteins unable to be transported away from the tissue space. Also, high levels of IgM and IgA indicated on the existence of inflammation, so did the relatively high C3 level. These findings constitute a clear indication for the decompressive therapy (11).

No abnormalities in enzyme concentration, with the exception of alkaline phosphatase, were found. This finding remains unclear. Calcium level was maintained at normal level,

Tab. 4 Concentration of electrolytes in leg lymph of 5 patients with obstructive (postsurgical and postirradiation) and 5 patients with primary lymphedema. Mean values of at least 3 measurements in each patient ± 1 S.E.

	Type of Lymphedema			
	Obstructive		Primary	
	mEq/l	L/S	mEq/l	L/S
Ca^{++}	7.26 ± 0.3	0.77 ± 0.03	6.56 ± 0.67	0.62 ± 0.11
Ca^{++}/g of protein	4.71 ± 0.28	3.24 ± 0.2	3.67 ± 0.24	2.86 ± 0.32
K^+	4.17 ± 0.05	0.91 ± 0.06	4.4 ± 0.27	0.88 ± 0.09
Na^+	140.7 ± 0.5	0.96 ± 0.01	145.7 ± 3.9	0.96 ± 0.03
Cl^-	101 ± 2.5	1.1 ± 0.03	106.4 ± 4.3	1.1 ± 0.05

such were the levels of other electrolytes and lymph constituents. This indicates that, in contrast to the proteins, the bloodlymph exchange of small molecular substances remained unaltered.

In summary, low levels of proteins, among them those active in the process of local immune defence, in peripheral lymph of patients with malignancies, irrespective of whether due to hypoproteinemia or imbalance between in- and outflux of proteins to the tissue space, may weaken host local response against foreign antigens. Also transport of some protein-bound drugs may be impaired. Lymphedema complicating surgery and radiotherapy of some tumors predisposes to the development of chronic latent infection, further disabling the patients. This is most likely due to the low lymph concentration of protein and interruption of the afferent arc of immunization following blockage of the lymphatic pathways.

The preliminary observations from this heterogenous group of patients indicate that the very scattered values of serum and lymph concentration of proteins may be attributed to at least 2 factors: stage of the disease, and effects of therapy on the vascular permeability for proteins. The latter awaits special investigations in selected group of patients and appropriate controls.

Acknowledgments

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