Lymphology 11 (1978) 170–173

C1q-Binding Substances in Peripheral Lymph in Bronchial Carcinoma

H.E. Heier, J.A. Sokolowski, Ø.P. Solheim

From The Norwegian Radium Hospital, Laboratory for Hematology and Lymphology, and General Department, Oslo 3, Norway

Summary

Peripheral leg lymph has been studied for the presence of C1q-binding substances by the ^{125}I-C1q binding radioassay in six male patients with untreated bronchial carcinoma. In serum, this assay is highly specific for antigen-antibody complexes (immune complexes). Three of the patients had immune complexes in serum, and they all also had C1q-binding substances in peripheral lymph. The C1q-binding activity was quite similar in serum and peripheral lymph. In the other three patients, immune complexes were not found in serum, but in one of them, moderate amounts of C1q-binding substances were found in peripheral lymph. The results suggest that immune complexes may be found in peripheral lymph in cancer patients in about the same amounts as in serum. However, formal proof is lacking that the C1q-binding substances of peripheral lymph are real immune complexes.

Complexes of antigen and antibody are frequently referred to as immune complexes (IC). In their presence, the activity of killer cells with Fc receptors (K cells and macrophages) can be blocked non-specifically. IC can also block antigen-specifically T lymphocyte cytotoxicity against membrane-bound antigen also present in the IC (1). IC have recently been detected in sera of a considerable number of cancer patients (2, 3, 4, 5, 6), and their biological role is presently subject to intense investigation.

IC can be detected in biological fluids by a variety of methods (1, 7). Some of these are based on the ability of complement factor C1q to bind IC. The modified ^{125}I-C1q-binding radioassay of *Zubler* et al (8) has proved to be highly specific for IC in both neoplastic and non-neoplastic diseases (1, 5, 7, 9). We designed the present investigation to see if signs of IC, as provided by this assay, can be found in peripheral lymph of cancer patients, and to provide some preliminary data on the relations between the IC concentrations in serum and peripheral lymph.

Patients and Methods

Patients. Six male patients with biopsy-proven bronchial carcinoma were investigated. None of them had received radio- or chemotherapy prior to investigation.

Control individuals. Ten healthy men served as controls for the lymph values. Control sera were obtained from 48 healthy blood donors.

Lymph drainage. A superficial leg lymph vessel was cannulated according to the method described by *Engeset* et al (10), without using patent blue injection prior to cannulation.

Lymph and serum sampling. On the third day after the insertion of the lymph cannula, peripheral lymph was collected without the presence of heparin or other anticoagulants between 8 A.M. and 3 P.M. During the period of lymph sampling, all persons investigated carried out moderate physical activity and were not in bed. After collection, the lymph was centrifuged for 15 min. at 1500 G and room temperature, and the supernatant was stored at –70 °C. Ten ml of whole blood were drawn at the end of the lymph sampling period, left at room temperature for 30–90 minutes, and then treated as the lymph. Serum was stored in the same way as the lymph.

^{125}I-C1q-binding radioassay. The assay was applied similarly in serum and lymph, as described by *Zubler* et al (8). Additional protein was not added to the lymph before testing. Sensitivity curves were produced for the assay in serum and lymph by adding increasing amounts of heat aggregated human immunoglobulin (*Kabi*, Sweden) to normal AB serum or to normal peripheral lymph, rendering a final volume of each sample of 100 µl, and then applying the test as described above. Briefly, the assay is based on the precipitation of IC which have bound ^{125}I-C1q at 4 °C using 3% polyethylen-

0024–7766/78 1600–0170 $ 04.00

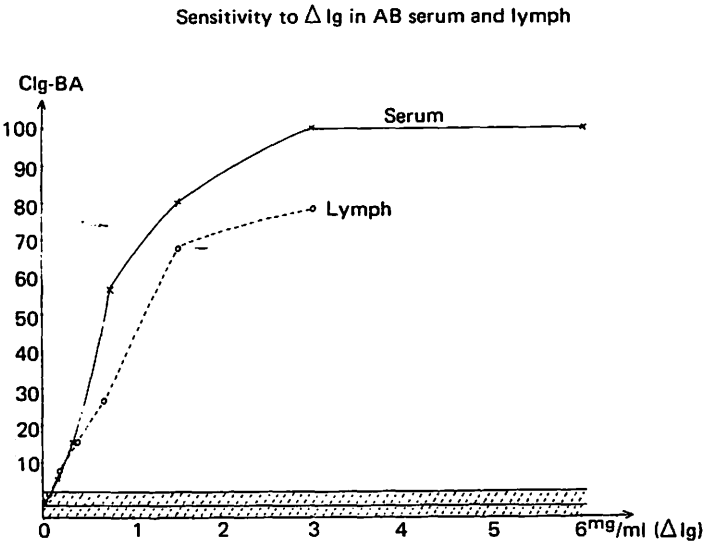

Fig. 1 Sensitivity of the ^{125}I-C1q-binding radioassay for various concentrations of heat aggregated human immunoglobulin added to normal human AB serum or normal human lymph. The curves are from a single experiment.

glycol. Results were expressed as the percentage of ^{125}I-C1q precipitated as compared to the total amount of radioactivity added. The results were corrected for "non-specific" precipitation of ^{125}I-C1q observed by testing normal sera, using a modification of Farr's formula (10). The corrected percentage of ^{125}I-C1q precipitated, represented the C1q-binding activity (C1q-BA) of the sample. The C1q-BA's of at least 5 normal sera were measured in each test. Results obtained in lymph, were corrected by the "non-specific" precipitation measured in serum. The normal range of C1q-BA was considered as the mean C1q-BA ± 2 SD of the 48 normal sera tested in the study. The normal range of C1q-BA for serum was found to be 0 ± 2%. Lymph and serum samples with C1q-BA exceeding 2.0%, were termed positive.

Results

Sensitivity of the C1q-binding radioassay in serum and lymph.

The limit of sensitivity was found to be 0.05 mg/ml of aggregated Ig in both fluids (fig. 1). With increasing concentrations of aggregated Ig, the C1q-BA possibly increased somewhat slower in lymph than in serum, but C1q-BA's of 0–20% appeared to correspond to about the same concentrations of aggregated Ig in both fluids.

C1q-BA in serum and lymph in patients and controls.

These values are shown in fig. 2. Positive sera were found in three of the patients. All of these also had positive lymph. One of the patients with negative serum had positive lymph. Two patients had both negative serum and lymph. The control individuals all had negative sera and lymph samples, except one, whose lymph was slightly positive.

Discussion

Previously strong indications have been presented that positive C1q-BA in sera from cancer patients reflects the presence of IC (5). Similar indications are lacking for peripheral lymph, and, although the present assay is considered highly specific for IC (1), it cannot be excluded that other substances with C1q-binding properties may have influenced the results in peripheral lymph.

Immunoglobulins and complement factors are present in peripheral lymph in 7–25% of their corresponding serum concentrations, depending on their molecular weight (11). IC in serum are larger than the IgG molecule, and not infrequently even larger than the IgM molecule (5). If the lymph samples are rendered positive by real IC, it may seem surprising to find that the C1q-BA of peripheral lymph is quite similar to that of serum, and that it may possibly be even

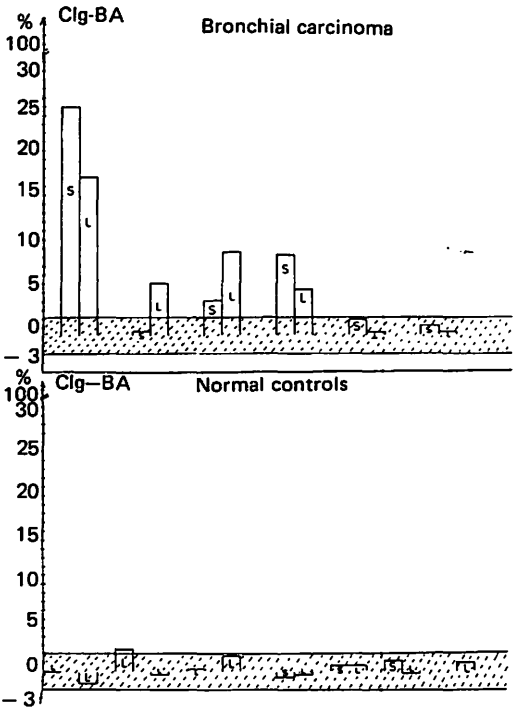

Fig. 2 C1q-binding activity (C1q-BA) in serum (S) and peripheral lymph (L) of six men with untreated bronchial carcinoma (upper part of figure), and in peripheral lymph of ten healthy men and serum in three of them (lower part of figure). Hatched areas show normal range for sera as defined by testing of 48 normal blood donors.

higher in peripheral lymph than in serum in some cases. This could be explained if the IC have a higher affinity for the capillary wall than have other serum proteins. Alternatively, it may be that antigen and antibody traverse the capillary wall separately and recombine in the tissue fluid, thus adding to the lymph C1q-BA brought about by IC which pass the wall as IC. A similar mechanism is known to operate in serum sickness (1).

If peripheral lymph is rendered positive by other substances than IC which can bind C1q, it is nevertheless of interest that these substances are found in cancer patients and not in normal controls. The nature of these substances should therefore be further investigated. If they should turn out to be real IC, the present study strongly indicates that factors blocking various cytotoxic processes, may easily gain access to the tumor site, even if formed in other parts of the body. Furthermore, since some patients may possibly be positive in lymph and negative in serum, it may not be relevant to base studies of the clinical role of IC in cancer solely on IC determinations in serum.

Acknowledgements

Hans Erik Heier is a fellow of the Norwegian Cancer Society. Ms. Mette Stinessen provided excellent technical assistance.

References

1 *World Health Organization:* The role of immune complexes in disease. Wld. Hlth. Org. Techn. Rep. Ser. 606 (1977)
2 *Theophilopoulos, A.N., C.B. Wilson, F.J. Dixon:* The Raji cell radioimmune assay for detecting immune complexes in human sera. J. clin. Invest. 57 (1976) 169–182
3 *Carpentier, N.A., G.T. Lange, D. Fiere, G.J. Fournie, P.H. Lambert, P.A. Miescher:* Clinical relevance of circulating immune complexes in human leukemia. J. clin. Invest. 60 (1977) 874–884
4 *Amlot, P.L., J.M. Slaney, B.D. Williams:* Circulating immune complexes and symptoms in Hodgkin's disease. Lancet 1 (1976) 449–451
5 *Heier, H.E.; N.A. Carpentier, G.T. Lange, P.H. Lambert, T. Godal:* Circulating immune complexes in patients with malignant lymphomas and solid tumors. Int. J. Cancer 20 (1977) 887–894
6 *Samayoa, E.A., F.C. McDuffie, A.M. Nelson, V.L. W. Go, H.S. Luthra, H.W. Brumfield:* Immunoglobulin complexes in sera of patients with malignancy Int. J. Cancer 19 (1977) 12–17
7 *Zubler, R.H., P.H. Lambert:* Immune complexes in clinical investigation. In Thompson, R. (ed.): Recent advances in clinical immunology, no. 1. Churchill Livingstone, Edinburgh, London and New York (1977)
8 *Zubler, R.H., G. Lange, P.H. Lambert, P.A. Miescher:* Detection of immune complexes in unheated sera by a modified ^{125}I-C1q-binding test. J. Immunol. 116 (1976) 232–235
9 *Nydegger, U.E., P.H. Lambert, H. Gerber, P.A. Miescher:* Circulating immune complexes in the serum in systemic lupus erythematosus and carriers of hepatitis B antigen. J. Clin. Invest. 54 (1974) 297–309
10 *Engeset, A., B. Hager, A. Nesheim:* Studies on human peripheral lymph. Lymphology 6 (1973) 6–12
11 *Olszewski, W.L., A. Engeset, H. Lukasiewicz:* Immunoglobulins, complement and lysozome in leg lymph of normal men. Scand. J. clin. Lab. Invest. 37 (1977) 669–674

H.E. Heier, Norwegian Radium Hospital, Laboratory for Hematology and Lymphology and General Department, Oslo 3, Norway

Discussion

Szabó: Could the occurence of immune complexes in cancer be secondary to the inflammatory reactions, necroses and so on, and not specific to antigens present on the malignant cells?

Heier: Immune complexes are by no means unique to cancer. Immune complexes may be present especially in rheumatoid and infectious diseases. Dr. Svěhag in Odense and his collaborators have recently found an increased C1q-binding activity in the acute phase of heart infarction, thus possibly relating the occurrence of immune complexes to inflammation. Accordingly, we may very well be dealing with an unspecific phenomenon. Our hope is, however, that some or all of these immune complexes will turn out to contain tumor specific antigens. There are some studies from melanoma and from Hodgkin's disease which do suggest the presence of such antigens in the immune complexes, but these studies are based on the use of cell lines and have not yet been confirmed.

Lassen: The local lymph from the tumor might be the best place to find something more specific. Have you studied local lymph from the tumor?

Heier: No, but we have planned to collect ductus thoracicus lymph from patients with gastrointestinal cancers.