

Immunohistochemical analysis of CD31, CD36, and CD44 antigens in human omentum

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ABSTRACT

Objective: Milky spots in the human omental tissue are known to be consisting of lymphocytes, macrophages and mast cells. Our goal was to evaluate the relationship of lymphoid cells and macrophages with vasculature and stromal components.

Methods: In this study we examined the biopsy specimens obtained from the adult patients whom were operated for different purposes in the General Surgery Department of Dicle University Hospital, Ankara, Turkey. We used CD31 as an endothelial cell marker, CD36 which is known to react with microvascular endothelium and adipocytes, and CD44 which is a hyaluronic acid receptor using an indirect immunoperoxidase technique.

Results: We observed that CD31 was mainly reactive with vascular endothelial cells and platelets, CD36 was reactive with microvascular endothelium and adipocytes and CD44 was mainly expressed by the endothelial cells of high endothelial venules, fibroblasts in stromal compartments and by large mononuclear cells.

Conclusion: We determined the structural and immunophenotypic features of omental lymphoid tissue components stressing vascular and stromal elements, and we briefly discussed the significance of the expression of these molecules in the determined locations.

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The omentum associated lymphoid tissue (OALT) was observed by Von - Recklinghausen in 1863 for the first time as white spots in the omentum of the young rabbits, and he had named such spots as "Milchflecke" (Milky Spot). In his study, Von Recklinghausen¹ defined these milky spots as intensive gathering of the connective tissue cells surrounding the capillary web.² The presence of the lymphoid tissue within omentum was established by the studies performed earlier in various types.³⁻⁶ The presence of OALT in humans was initially discovered by Seifert.⁷ On the fresh samples obtained from the surgical operations, Shimotsuma et al⁴ confirmed the histological and anatomic structure of the milky spots in human. Borisov⁸ investigated the milky spots in the human omentum at the tissue samples obtained from

autopsy, and stated that there were milky spots present at the omentum of the fetus, but that the number and intensity of such spots decreased during life span. Kampmeier⁹ identified the milky spots at the parietal pleura adjacent to the aorta of the new born infants. It is suggested that the omental milky spots of the experimental test animals displayed similarities with the omental milky spots present in human.^{10,11} Trebichausky et al¹² conducted studies on the pigs as experimental test subjects and reported that the development of the omental milky spots were closely related with the general immunological condition of the animals. Although the definite function of the milky spots in humans is not clearly known, they have been identified to strongly react with the intraperitoneal antigens. Several studies had defined the function of the macrophages present

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within the milky spots to clear the microorganisms and especially the cancerous cells of the peritoneal cavity.^{13,14} In addition, it has been suggested that the milky spots could be the source of a microenvironment that provides the peritoneal macrophages from the macrophage pioneer cells possibly deriving from the bone marrow are locally produced.¹⁵⁻¹⁷

The aim of the present study was to determine the stromal components and lymphoid elements of omental tissue to obtain further evidence on its structure and function immunohistochemically. Monoclonal antibodies specific to antigens expressed by stromal elements and vasculature (CD31, CD36 and CD44) are used for this purpose.

Methods. In order to examine the milky spots within the human omentum tissue, the tissue samples were taken from 20 patients with their full consent who underwent an operation under selective conditions with the pre diagnosis of cholecystitis at the Department of General Surgery, Medical Faculty of Dicle University, Ankara, Turkey, during 1999 through to 2001. The tissue samples were immediately frozen in liquid nitrogen at -196°C and were kept in a -30°C deep freeze until use. Seven micrometer thick serial sections were cut using a cryostat, and these sections were mounted on gelatin covered microscopic slides. After drying at room temperature, the samples were kept in humidity free containers with silica gel (Merck, 1.01925) until immunostaining has performed. An indirect immune peroxidase technique was used as previously described by Dijkstra et al.¹⁸ Used as primary antibodies were anti-human CD31 monoclonal antibody (Santa Cruz, cat. no: SC-1505, California (CA), United States of America (USA)); anti-human CD36 monoclonal antibody (Santa Cruz, cat. no: SC-9154, CA, USA) and anti-human CD44 monoclonal antibody (Sigma, cat. no. C-7923, Saint Louis, USA). As secondary antibody, 1:200 rabbit anti - mouse IgG peroxidase (Sigma, cat. No: A - 9044, Saint Louis, USA) diluted in PBS/BSA and 1:100 normal human serum solution was used. Control staining was performed using irrelevant mouse monoclonal antibodies and omitting the primary antibody step. Sections were examined and photographed by Olympus BH2 light microscope.

Results. The findings of the omentum tissue samples are summarized as follows:

Controls. There was no specific staining in control slides. Several granulocytes with in the section plane were strongly reactive as a result of the endogenous peroxidase reactivity (**Figure 1a**).

CD31 reactivity. The CD31 antigen was expressed mainly by the endothelial cells of all types of vasculature including high endothelial

venules (**Figure 1b**). Intensive expression of the antigen on vascular endothelial cells revealed the vascular network clearly. At higher magnifications the endothelial membranous reaction was more prominent delineating individual endothelial cells. Few platelets present in the lumina of vasculature were also reactive for the antigen (**Figure 1c**). The endothelial cells of the high endothelial venules located within the milky spots in small numbers were also determined to be strongly expressing the CD31 (**Figure 1d**).

CD36 reactivity. In the sections immunostained with anti-CD36 monoclonal antibody we have determined that the endothelial cells belonging to large arteries and veins are not reactive for this antigen. A weak diffuse staining was present in the vascular smooth muscle layer of these vessels. At higher magnifications, a strong reaction confined to endothelial cells of high endothelial venules was detected (**Figure 1e**). A prominent reaction for CD36 antigen was also present in the adipose tissue on adipocytes and blood vessels of smaller diameter (**Figure 1f**).

CD44 reactivity. Main reactivity for CD44 antigen was determined in stromal components including fibroblasts and vascular endothelial cells. An interesting finding was the prominent membranous CD44 reactivity on large mononuclear cells. These cells were sometimes occurring in groups. Examination of strongly CD44 reactive areas at higher magnifications revealed that at least some of these areas were containing high endothelial venules while in other areas an epithelium like arrangement of cuboidal cells was evident (**Figure 1g**). To clarify the reactivity pattern we examined serial sections of the same specimens and we concluded that most of these epithelioid clusters were belonging to tangential sections of high endothelial venules. Especially in those cells which are individually determined scattered with in the tissue the membranous pattern of reactivity was more prominent (**Figure 1h**).

Discussion. Structurally omentum is composed of a loose connective tissue rich in adipose component enclosed by peritoneum. Within the stroma, a number of lymphoid elements are also present including small lymph nodes and lymphoid aggregates along vasculature. Such aggregates are termed as milky spots or omentum associated lymphoid tissue and the main function of this tissue is suggested to be clearing the peritoneal cavity from invaders including bacteria; in other words, the tissue is responsible from the defense of the peritoneal cavity. Besides, it has been suggested that the lymphocytes or the macrophages stimulated within this tissue recycle and reach to the secondary lymphoid organs and assist in the development of an

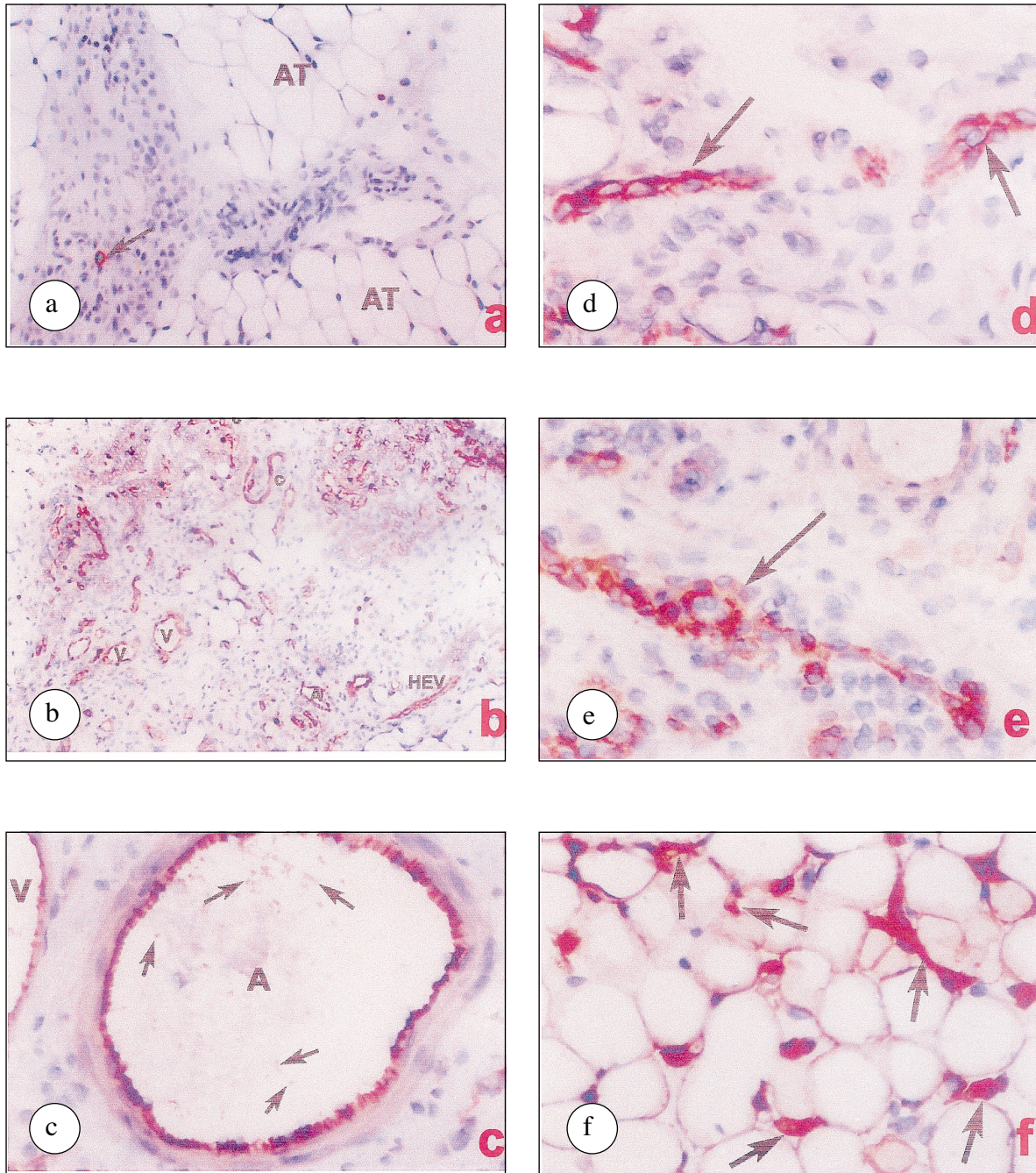


Figure 1 - (a) A milky spot embedded within the adipose tissue (AT) in an omentum section is seen. A granulocyte located within the lymphoid tissue was intensively stained (arrow). Indirect immunoperoxidase (IP) - hematoxylin counter stain (HCS) x 20. (b) Low power micrograph of omental tissue containing several blood vessels of different types. Endothelial cells of all types of vasculature (A - artery, V - Vein, C - capillary, HEV - high endothelial venule) were CD31 (+). IP-HCS x 10. (c) CD31 reactivity is seen in a section through a medium sized artery (A) and vein (V). Few platelets were also reactive for CD31 antigen (arrows). IP-HCS x 40. (d) High endothelial venules (arrows) is observed. Strong CD31 reactivity on endothelial cells is seen. IP-HCS x 40. (e) Strong CD36 reactivity is observed in high endothelial venule (arrow) located at the omental lymphoid tissue. IP-HCS x 40. (f) CD36 reactivity in an area rich in adipose tissue is seen. Reactivity on capillaries is relatively much stronger (arrows) when compared with the moderate CD36 reactivity on adipocytes. IP-HCS x 40.

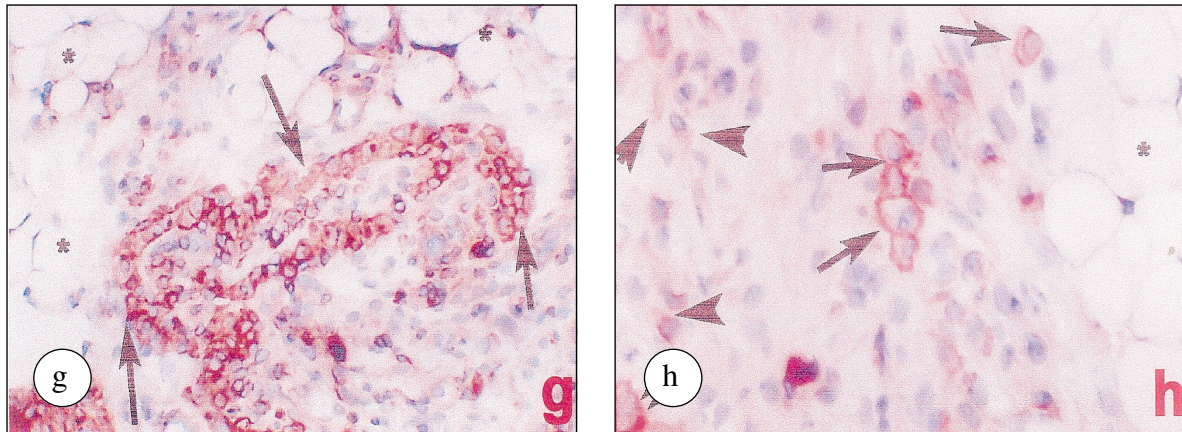


Figure 1 - (g) Section through a milky spot and adipose tissue (*). Epitheloid cell masses (arrows) were strongly CD44(+). IP-HCS x 20. CD44 reactivity in a section through lymphoid and adipose (*) compartments of omentum. Some fibroblasts (arrow heads), were moderately reactive. Large mononuclear cells occurring individually or forming small clusters (arrows) exhibiting a membranous CD44 reactivity pattern. An area belonging to one of the larger masses is visible (double arrow). IP-HCS x 40.

efficient immunity response versus certain antigens. Dux et al¹⁰ and Beelen⁵ defined the omental lymphoid tissue as effective secondary lymphoid organs and suggested that these tissues are involved in the production of numerous cells involved in submitting antigen to the lymphocyte pool within circulation. However, Van Vugt et al³ who carried out studies on omental lymphoid tissue did not agree that the milky spots, on view are secondary lymphoid organs. Our study shows that the immunohistochemical examination of omental tissue revealed the vascular and lymphoid elements clearly. Aggregates of lymphoid cells and macrophages were found in all tissue samples examined in addition to few small lymph nodes which are distinguished easily by their capsules and germinal centers. An extensive network of vasculature was revealed by anti-CD31 and anti-CD36 monoclonal antibodies reflecting the highly dynamic nature of the tissue. Groups of lymphocytes and macrophages were usually observed along the vasculature especially around post capillary venules with high endothelial cells. All 3 antigens were strongly expressed in high endothelial venules. As well documented previously¹⁹⁻²¹ these vasculature represent a specific site for lymphocyte entry to tissues. Thus, existence of numerous high endothelial venules in the tissue reflects the rate of recirculating lymphoid cells to the tissue. In our view this finding suggests omentum associated lymphoid tissue is an active secondary lymphoid tissue. Additionally, CD44 has been defined as one of the most important homing molecules that arrange the migration of

lymphocytes from the high endothelial venules to the tissues and CD31 functions as an adhesion molecule that acts in the terminal phase of the migration process.²² Expression of CD31, CD36 and CD44 on these high endothelial cells suggest that these antigens participate in trafficking of leukocytes, especially lymphocytes to the tissue. Parallel to our consideration, Mironov et al²³ observed that the milky spots located on the human omentum acts as the primary actor in the defense of the peritoneal cavity in his study absorbing the foreign particles including the cancerous cells. These authors also suggested that milky spots undertake a focus role in the metastasis of cancer cells.

In previous reports^{24,25} on the omental lymphoid tissue some investigators made comparisons in order to classify the milky spots according to their components. Renault²⁶ classified the milky spots into 2 whether they contain or lack vascular component. Although we determined a major size difference between the lymphoid tissue accumulation depending on the lymphoid cell and macrophage numbers, we observed that even the smallest lymphoid aggregates contain a vascular component, mostly high endothelial venules thus, Renault's²⁶ classification is somehow misleading and possibly arising from insufficient detection of vascular components examined.

In a similar manner, Imai et al²⁷ classified the milky spots into 2 groups depending upon the presence of the lymphatic follicles: type-1 milky spots lacking follicles and type-2 milky spots having follicles. In our study we did not detect any follicles

within lymphoid aggregates. Lymphatic follicles and germinal centers were present only in the small lymph nodes embedded with in the tissue. In sections, some of these lymph nodes appear as small aggregates of lymphoid cells with follicles especially in sections passing through the peripheral narrower compartments of lymph nodes possibly resulting in a misleading interpretation as even though the sections through lymph nodes were small they are always surrounded by a capsule distinguishing these structures (with follicles) from lymphoid aggregates along vasculature (lacking follicles). We strongly believe that lymphoid aggregates along vascular channels in omentum lack lymph follicles like in the dermal lymphoid tissue.

In previous studies, Shimotsuma et al² and Borisov⁸ described extensive glomerule like-capillary networks. We also agree that omental tissue is extensively rich in vasculature including capillaries and veins. Using monoclonal antibodies reactive with vascular endothelium these vasculature are revealed more clearly. Expression of CD31, CD36 and CD44 in omental tissue is not reported in detail previously. In this respect, our findings will be valuable as preliminary data for future directions. Additionally, we determined large mononuclear cells with a membranous CD44 reactivity. In serial sections, some of these cells were found to belong to high endothelial venule lining. Most of these cells had a large cytoplasm with a relatively euchromatin rich nucleus. However, some of these cells reflected the structural features of phagocytic cells but usually lacking heterophagosomes.

In conclusion, these cells are not typical macrophages and described these cells as large mononuclear cells keeping in mind that they might represent a special subgroup of lymphocytes (possibly B1 cells). We believe that further phenotypic analysis directed to these cells will help to distinguish these cells clearly.

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