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Comparative immunohistochemical staining of atherosclerotic plaques using F16, F8 and L19: Three clinical-grade fully human antibodies

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ABSTRACT

Objective: F16, F8 and L19 are three fully human monoclonal antibodies, specific to splice isoforms of tenascin-C and fibronectin, which stain sites of active tissue remodeling and which are currently in Phase I and II clinical trials as radio-immunoconjugates and immunocytokines in patients with cancer and arthritis. The characterization of atherosclerosis using these antibodies may open novel pharmacodelivery options for the imaging and treatment of cardiovascular conditions. It may also allow a better assessment of the corresponding immunoconjugates in polymorbid patients with atherosclerotic plaques.
Methods: We performed a comparative immunohistochemical analysis with the F16, F8 and L19 antibodies in 28 freshly frozen human carotid plaques and in 11 normal arteries. Furthermore, we assessed the localization of the antibodies in relation to the infiltrating macrophages, *vasa vasorum* and Ki67-positive proliferating cells of the plaque.
Results: The F16 antibody, specific to the extra-domain A1 of tenascin-C, stained plaques with a selective and intense pattern, while F8 and L19, specific to the EDA and EDB domains of fibronectin, respectively, exhibited a less selective and intense staining. In immunofluorescence, F16 was found to bind regions rich in macrophages, *vasa vasorum* and proliferating cells, while showing no detectable *vs.* weak staining of normal arteries and of quiescent plaque structures.
Conclusion: The human monoclonal antibody F16 stains areas of active tissue remodeling in atherosclerotic plaques and may thus deserve to be investigated as a suitable building block for the development of radiopharmaceuticals for plaque imaging or for the antibody-based targeted delivery of therapeutic agents to atherosclerotic lesions.

1. Introduction

The use of monoclonal antibodies for the pharmacodelivery of therapeutic agents (e.g., drugs, cytokines, radionuclides, photosensitizers, pro-coagulant agents) at sites of disease is becoming an attractive pharmaceutical modality in the field of cancer and chronic inflammatory disorders, with the aim to develop drugs that act at the site of disease while sparing normal tissues [1–4].

F16, F8 and L19 are three fully human monoclonal antibodies developed by our laboratory, which recognize markers of angiogenesis and of tissue remodeling and which are currently used for the pharmacodelivery of radionuclides or cytokines in patients with cancer and arthritis [5–14]. F16 recognizes the alternatively-spliced A1 domain of tenascin-C, while F8 and L19 recognize the alternatively-spliced EDA and EDB domains of fibronectin, respectively [15–17]. These components of the modified extracellular matrix are known to be virtually undetectable in normal human tissues (exception made for the endometrium in the proliferating phase), but to be over-expressed during fetal development and at sites of active tissue remodeling, with a vascular and/or stromal pattern of staining [18]. Seven derivatives of F16, F8 and L19 [F16-IL2, F16-^{131}I, F16-^{124}I, F8-IL10, L19-IL2, L19-^{131}I, L19-TNF] are currently being investigated in Phase I and Phase II clinical trials in cancer and in rheumatoid arthritis [1,14,19].

Tissue remodeling and angiogenesis (e.g., *vasa vasorum*) are important processes during atherosclerotic plaques formation [20,21]. Indeed, we have previously shown that monoclonal antibodies to splice isoforms of tenascin-C and fibronectin can

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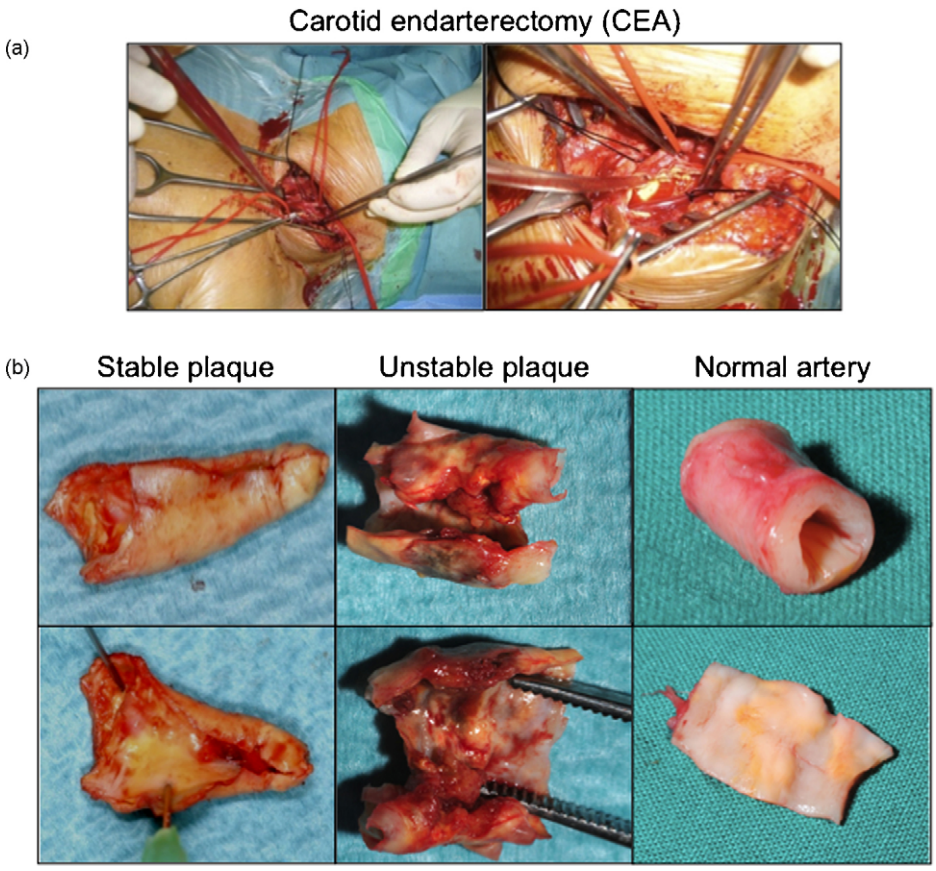

Fig. 1. (a) Pictures of a carotid endarterectomy (CEA) performed in the Clinic for Cardiovascular Surgery of the University Hospital of Zurich. (b) Left, up and down: pictures of a stable atherosclerotic plaque. Middle, up and down: pictures of an unstable atherosclerotic plaque. Right, up and down: segment of a normal external iliac artery.

selectively localize at atherosclerotic plaques *in vivo* following intravenous administration, using ApoE knock-out mice fed with a cholesterol-rich diet as animal model [22,23]. A direct comparative immunohistochemical analysis of the F16, F8 and L19 antibodies in human atherosclerotic plaques has not been reported so far. Such studies are complicated by the fact that these antibodies do not work well in formalin-fixed, paraffin-embedded specimens, but rather require the analysis of freshly frozen tissue [24,25]. A detailed knowledge of the ability of the three antibodies to react with atherosclerotic plaques in patients would be important not only in view of possible imaging and pharmacodelivery applications in the cardiovascular field [26], but also in consideration of the fact that these clinical-stage antibodies may be administered to polymorbid cancer patients and may thus target cytokines or radionuclides to sites of atherosclerosis. In spite of promising results with the L19-based targeted delivery of interleukin-2, which was reported to reduce atherosclerotic plaques in the ApoE−/− mouse model [27], at this moment in time we do not have a detailed knowledge of whether the antibody-based targeted delivery of pro-inflammatory (e.g., IL-2, TNF) or anti-inflammatory (e.g., IL-10) cytokines to atherosclerotic plaques may have a beneficial or detrimental effect.

In this article, we describe a comparative analysis of the F16, F8 and L19 antibodies in 28 freshly frozen carotid plaques and in 11 normal arteries, using immunohistochemical and immunofluorescence procedures. The best staining results were obtained with the F16 antibody, which was found to strongly react at certain areas of plaques rich in macrophages, *vasa vasorum* and proliferating cells, while showing no detectable *vs.* weak staining of normal arteries and of plaque areas not involved in active remodeling.

2. Materials and methods

2.1. Subjects' characteristics

The study group comprises 28 carotid atherosclerotic plaques, collected during carotid endarterectomy (CEA) from 20 patients, and 10 fragments of normal external iliac artery from patients treated for abdominal aortic aneurysm. Also a normal pulmonary artery from the lobectomy specimen of a patient who was operated for non-small cell lung cancer was used. Tissue specimens were immediately processed according to the guidelines of the Swiss Society of Pathology for frozen sections. Plaque or normal artery fragments were snap-frozen in OCT (optimal cryo-temperature) medium and stored at −80 °C. Data collected included age, gender, clinical history, macroscopic evaluation at the moment of surgery and classification into stable and unstable plaques.

2.2. Antibodies

The F16 antibody, specific to the extra-domain A1 of tenascin-C, the F8 antibody, specific to the extra-domain A of fibronectin, and the L19 antibody, specific to the extra-domain B of fibronectin, have been described before [15–17].

2.3. Immunohistochemistry

For immunohistochemistry, the F16, F8, and L19 antibodies were used in biotinylated small immunoprotein (SIP) format [16,17,28,29]. Aliquots of antibodies were prepared from a single batch, stored at 4 °C, and were used only once, thus contributing to excellent reproducibility of immunohistochemical results [30].