INTRODUCTION

During the process of acute inflammation, circulating polymorphonuclear leukocytes (PMN) transmigrate from the vascular lumen to the site of infection or injury. This process involves the interaction of PMN with endothelial cells (EC) and subsequent diapedesis of PMN through the subendothelial basement membrane (BM). Along the path of transmigration, PMN come across and interact with numerous macromolecules of BM. Extensive research has been carried out to understand how various components of the BM e.g. type IV Collagen (COL (IV)), laminin, entactin, and proteoglycans mediate the physiological function of PMN (Matzner, Bar-Ner, Yahalom, Ishai-Michaeli, Fuks, and Vlodavsky, 1985; Matzer, Vlodavsky, Michaeli, and Eldor, 1990; Pike, Wicha, Yoon, Mayo, and Boxer, 1989; Senior, Hinek, Griffin, Pipoly, Crouch, and Mecham, 1989; Senior, Gresham, Griffin, Brown, and Chung, 1992). Studies by Huber and Weiss (1989) suggest that the transmigrating PMN cause a transient focal disruption of the BM which allows PMN to traverse it. These disruptions are rapidly repaired by the overlying EC. In our laboratory, the focus has been on the role of COL (IV), a major component of the BMs, on PMN function.

COL (IV) is a heterotrimer molecule composed of three α chains. The predominant molecular species is [α1(IV)]2 α2(IV). The presence of additional chains α3(IV), α4(IV), α5(IV) and α6(IV) has been demonstrated (Hudson, Reenders, and Tryggvason, 1993; Ninomiya, Kagawa, Iyama, Naito, Kishiro, Seyer, Sugimoto, Ooahashi, and Sado, 1995). Although the genes for the various COL (IV) chains have been cloned, the exact molecular structure of the COL (IV) involving the new α-chains is not yet clear (Kamagata, Mattei, and Ninomiya, 1992; Mariyama, Leinonen, Mochizuki, Tryggvason, and Reenders, 1994; Zhou, Hertz, Leinonen, and Tryggvason, 1992; Zhou, Ding, and Reenders, 1994).
PMN produce superoxide $O_2^-$ and release proteolytic enzymes upon activation by various agents such as PMA, fMLP and even collagen type I (Monboisse, Bellon, Randoux, Dufer, and Borel, 1990). Previous In vitro studies from our laboratory have established that in contrast to type I collagen, COL (IV) does not activate PMN but instead inhibits their activation by PMA, fMLP and collagen type I (Monboisse, Bellon, Perreau, Garnotel, and Borel, 1991). The inhibitory activity was localized in the non-collagenous domain (NC1 domain) of the α3 chain. Additional studies using synthetic peptides showed that the inhibitory activity resides within a sequence comprising residues α3(IV) 185-203 (Monboisse, Garnotel, Bellon, Ohno, Perreau, Borel, and Kefalides, 1994.) The N-terminal cysteine and the triplet -SNS- (residues 189-191) were absolute requirements for such activity.

In this study, we used an in vitro model of a vessel wall construct and examined the integrity of subendothelial BM after PMN, treated with various peptides of COL (IV), came in contact with it. The extent of the integrity of BM deposited over the collagen gel by EC was assessed by its resistance to the penetration of a colloidal pigment into the collagen gel. The results suggest that PMN treated with the α3(IV) 185-203 peptide from the NC1 domain of COL (IV) have an impaired ability to damage the subendothelial BM. The results also show that the triplet -SNS- (residues 198-191) of the α3(IV) peptide is essential for the observed effect. However, this ability to prevent damage to BM by nonactivated PMN was abolished when the experiment was carried out in the presence of the activator fMLP. We have noted that the PMN ability to damage was similarly reduced by an antibody to CD47 antigen which is present on both cell types and is involved in EC-PMN interaction. On the other hand, sequential treatment of PMN with anti-CD47 antibody followed by the α3(IV) 185-203 peptide abolished the effect of either ligand. This result is corroborated by unpublished observations from our laboratory indicating that the CD47 antigen in association with the αvβ3 integrin serves as part of the receptor complex for α3(IV) peptide.

2. MATERIALS AND METHODS

2.1. Cell Culture: Endothelial cells were isolated from human umbilical cord according to Gimbrone, Cotran and Folkman (1974) with some modifications. The cells were grown in tissue culture flasks coated with 1% gelatin and fed with modified M199 medium containing fetal bovine serum (20%), L-glutamine (2 mM), gentamicin (10 μg/ml), amphotericin B (2.5 μg/ml) and supplemented with heparin (90 μg/ml) and endothelial cell growth factor (30 ng/ml) (Ziaie, Friedman, and Kefalides, 1986).

2.2. PMN Preparation: Blood was obtained from healthy individuals and PMN were isolated through a Ficoll-Hypaque gradient. The PMN layer was collected, diluted with PBS and centrifuged two cycles to remove the gradient materials. The contaminating erythrocytes were lysed with a hypotonic solution (0.1x PBS). The cells were then washed three more times and finally resuspended in the appropriate medium.

2.3. Cell Culture in the Vessel Wall Model: Double chamber tissue culture dishes (Becton Dickenson, Franklin Lakes, N.J.) were used to grow EC on the collagen gel. Collagen gels were essentially prepared according to and Huber and Weiss (1989). Collagen type I stock solution was mixed with reconstitution buffer (0.05 M NaOH, 0.2 M Hepes, and 0.26 M NaHCO3), M199 medium and water to obtain a 2% collagen solution. NaOH (15-30 μl of 1 N) was added to adjust the pH. All solutions were kept at 4° C until the collagen solution was dispensed onto the inserts. The inserts (2.3 cm in diameter) with a polyester membrane (3 μ pore) were overlaid with 0.8 ml of ice-cold collagen solution and