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Structure, Regulation, and Pathophysiology of Tight Junctions in the Gastrointestinal Tract

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The tight junction, or *zonula occludens,* forms an intercellular barrier between epithelial cells within the gastrointestinal tract and liver and, by limiting the movement of water and solutes through the intercellular space, maintains the physicochemical separation of tissue compartments. The paracellular barrier properties of junctions are regulated and quite different among epithelia. The junction also forms an intramembrane barrier between the apical and basolateral membrane domains, contributing to segregation of biochemically distinct components of these plasma membrane surfaces. Here we briefly review three rapidly developing areas of medically relevant basic knowledge about the tight junction. First, we describe the presently incomplete knowledge of the molecular structure of the tight junction as a framework for understanding its functional properties. Second, we consider experimental evidence defining how the barrier properties of junctions are physiologically regulated and, third, how barrier properties are specifically altered in, and contribute to, pathologic processes affecting epithelia.

INTRODUCTION

Epithelia are composed of sheets of polarized cells capable of vectorial transport of water and solutes. The space between individual epithelial cells must be sealed so that transepithelial osmotic and electrical gradients are maintained. The structure which creates this paracellular barrier is the tight junction. Characteristics of the tight-junction barrier vary widely among different epithelia. The magnitude of the barrier, measured by transjunctional electrical resistance, varies over several orders of magnitude, creating so-called "leaky" epithelia such as that lining the gallbladder and "tight" epithelia such as those found in the stomach and colon [1]. Another variable property is the size of particles permitted to traverse the paracellular space between cells, typically in the range of 0.8 to 1.8 nm [2]. In combination with specific *transcellular* transport mechanisms, the *paracellular* barrier properties of tight junctions define the basic physiology of epithelia and, if altered, may significantly contribute to epithelial pathology. In this review, we consider what is presently known of the molecular structure of the tight junction, which is an emerging area of study and has already begun to contribute to our understanding of its functional properties. We then consider both the normal physiologic regulation of junctions as well as their alterations in pathologic states. We focus, for the most part, on

Abbreviations: CBD: common bile duct PKC: protein kinase C tBuBHQ: 1,4-benzohydroquinone ZO-1: *zonula occludens* one

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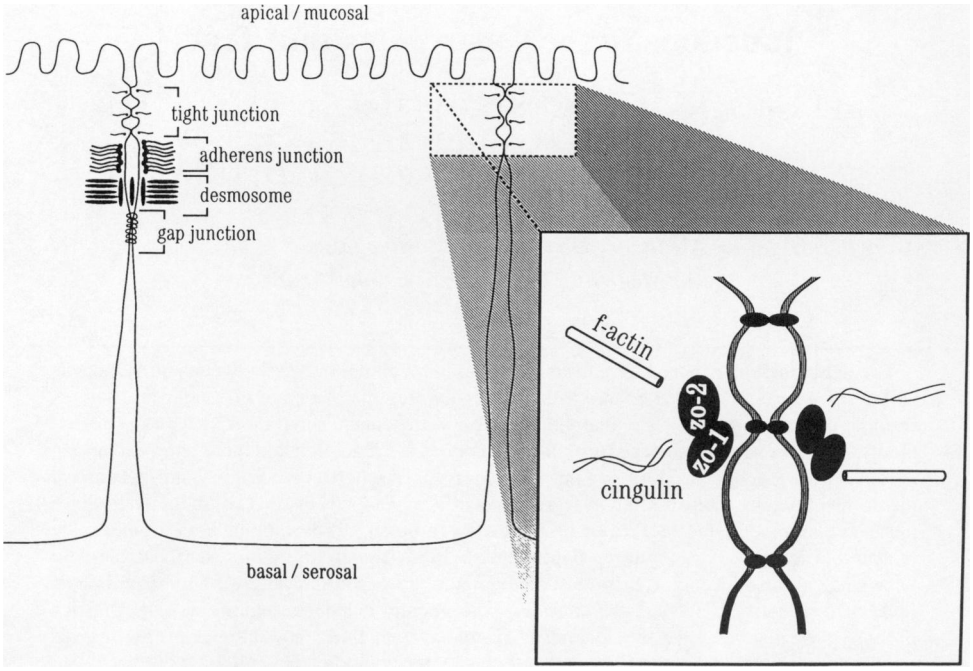

FIG. 1. Schematic diagram of the intercellular junctions of epithelial cells (*left*) and model of the molecular organization of the tight junction (*right*). Epithelial cells are joined by a recognizable set of intercellular junctions (see text for details). The apical most of these is the tight junction, which forms a continuous zone of intercellular contact around cells. The tight junction is formed by rows of contiguous transmembrane proteins which traverse the intercellular space and contact similar proteins on adjacent cells. A plaque of cytoplasmic proteins including ZO-1 (two isoforms), ZO-2, and cingulin are found along the regions of intracellular contact. Actin microfilaments enter the plaque and presumably link the tight junction with the cytoskeleton. ZO-1 and ZO-2 are known to associate physically; all other specific protein interactions are presently undefined. Bar signifies 20 microns.

physiology and pathology of the gastrointestinal tract; however, conclusions derived from these observations can probably be generalized to epithelia of other organs as well as the vascular barriers formed by tight junctions between endothelial cells.

Several excellent reviews exist of the individual topics covered here [1,3–6].

MOLECULAR STRUCTURE OF THE TIGHT JUNCTION

Light microscopic study of the epithelia during the late nineteenth century described a specialized region at the apical end of intracellular borders referred to as the "terminal bar" [7]. It was assumed that this region served an adhesive and barrier function between the individual epithelial cells. With the introduction of the electron microscope, this region of cell-cell contact was further defined to be composed of several different identifiable intercellular junctions (Fig. 1). Among these are desmosomes, which serve an adhesive function; gap junctions, which create intercellular pores, allowing communication of small molecules between adjacent cells; and adherens junctions, which are presently assumed to provide intercellular adhesion as well as being a prominent site for attachment of the actomyosin cytoskeletal [8,9]. Invariably the apical most of these intracellular junctions is the tight junction, or *zonula occludens*. Early electron microscopic studies demonstrated an absolute

intercellular barrier to diffusion at the tight junction, using electron-dense tracer molecules like colloidal lanthanum [10]. Coupled with static electron microscopic images, this finding led to the misconception of the tight junction as an absolute and non-regulated barrier. In cross-sectional images, the tight-junction region is composed of a variable number of points, or "kisses," at which the membranes of adjacent cells come into very close contact. Ultrastructural studies have demonstrated that actin microfilaments terminate on the membrane near these sites [11,12], although the actin in this region is quantitatively much less concentrated than at the adherens junction, where a dense circumferential ring of actin is associated with the membrane.

Ultrastructural details of the tight junction are better defined using the freeze-fracture electron microscopy technique. When frozen tissue is fractured, the cleavage plane tends to travel between the lipid bilayers of the plasma membrane. When the fractured surface is shadowed with platinum and viewed in the electron microscope, the zone of the tight junction appears as an anastomosing collection of fibrils within the membrane, circling the apical-basolateral boundary of the cell [13,14]. In tissue which is not fixed with aldehydes, the fibrils appear to be composed of rows of intramembrane particles, which are assumed to be transmembrane proteins. Sites of membrane contact correspond to these rows of transmembrane proteins, and it is further assumed that the homotypic interaction of these proteins between adjacent cells creates the intercellular resistive barrier. This assumption is further supported by the positive correlation between the number of fibrils and the paracellular electrical resistance observed among some epithelia [13,15].

Several components of the junction have recently been identified, all of which are peripherally associated membrane proteins present in a cytoplasmic plaque under the sites of cell-cell contact (Fig. 1). The first of these is ZO-1 (*zonula occludens* one), which is an approximately 220 kDa phosphoprotein, located specifically under the transmembrane particles [16–18]. Recent cDNA sequencing has demonstrated two isoforms of the ZO-1 protein that differ by an internal 80 amino acid domain referred to as motif-α, which appears to result from alternative RNA splicing [19]. The cell- and junction-specific distribution of ZO-1 isoforms (ZO-1α^+; ZO-1α^-) is presently undefined, but we speculate that differential expression may contribute to the great diversity in junction properties observed among tissues. A second junction-specific protein is cingulin [20], which is more abundant than ZO-1 and, through ultrastructural immunogold localization, appears to be located about three times as far from the membrane bilayer [18]. Rotary-shadowed electron microscopic images of purified cingulin demonstrate the molecule to be a highly extended dimer [21], and preliminary cDNA sequence information predicts it to contain alpha-helical structure homologous to that of tropomyosin and the tail of myosin II [22]. By analogy with these known cytoskeletal proteins, this finding suggests that cingulin may serve as a linker molecule between the subjunctional plaque and the cytoskeleton. A third junction protein, termed ZO-2, has been identified in cultured epithelial cells through its ability to bind and be immunoprecipitated with ZO-1 [23]. At the present time, there are no specific probes, such as antibodies and cDNAs, for its study; thus, its cellular and tissue distribution are undefined. Finally, a component referred to as the BG9.1 antigen has been described and shows an immunolocalization very similar to that of ZO-1 and cingulin; however, no subsequent reports of this protein have

followed [24]. Features of the molecular structure and components of the tight junction are presented schematically in Fig. 1.

PHYSIOLOGIC REGULATION OF THE TIGHT JUNCTION

The barrier properties of tight junctions vary among different epithelia and are regulated by a variety of stimuli within epithelia in response to physiologic demands [5]. The molecular mechanisms that bring this regulation about are not known, and interpretation of many results in this area remains problematic because changes in paracellular permeability have not been distinguished from changes in membrane conductance. Nevertheless, enough examples exist to suggest at least two general mechanisms for regulation of the permeability through the tight junction. First, hormones and other agents act through second-messenger pathways to affect directly junctional components, presumably through regulation of protein phosphorylation. The ultimate targets of kinase/phosphatase cascades remain unresolved, although both ZO-1 [17] and cingulin [25] are phosphoproteins. Second, alterations of the actin cytoskeleton could result in secondary changes in intercellular junction contacts. Some examples supporting each mechanism are cited below; these mechanisms are not mutually exclusive but may act together to alter tight junction permeability.

Both circulating hormones and neuronal influences can change paracellular resistance. In the perfused rat liver, vasopressin, epinephrine, and angiotensin II rapidly increase the tight-junction permeability [26]. Cholinergic stimulation of pancreas [27,28], parotid gland [29], and intestinal crypt cells causes penetration through the tight junction of normally excluded ultrastructural tracers. In the same studies, villus epithelial tight junctions were unaffected, suggesting a differential crypt to villus regulation [30]. There are fewer examples of hormones which "tighten" junctions, e.g., thyrotropin releasing hormone increases transepithelial electrical resistance in monolayers of cultured MDCK cells [32].

The Role of the Protein Kinase C Pathway

Vasopressin, angiotensin II, and epinephrine all act through changes in calcium, the inositol polyphosphates, and diacylglycerol. The effects of these hormones on paracellular resistance in the isolated, perfused rat liver can be mimicked by the use of the Ca^{++} ionophore, A23187 [33], and an inhibitor of microsomal Ca^{++} sequestration, 2,5-di-(tert-butyl)-1,4-benzohydroquinone (tBuBHQ [34]). Recently, two separate intracellular pathways have been proposed to explain the increase in tight junction permeability that follows the vasopressin-induced intracellular calcium increase in isolated hepatocytes. Yamaguchi et al. [35] demonstrated that vasopressin stimulates myosin light-chain phosphorylation, which is hypothesized to produce microfilament contraction. Nathanson et al. [36] have presented evidence that calcium acts by activation of protein kinase C (PKC), presumably by altering phosphorylation of proteins other than the myosin light chain or its kinase. Similar results have been demonstrated in the human intestinal T84 cell line, where an increase in paracellular permeability produced by a Ca^{++} ionophore can be blocked by two PKC inhibitors but not by a calmodulin inhibitor [37]. These observations are consistent with the involvement of the protein kinase C pathway in regulation of paracellular permeability.

Changes in Ca^{++} levels are not only important in the hormonal regulation of the tight junction but have been implicated in regulation of its assembly and disassembly.

Tight junctions are very sensitive to Ca^{++}; removal of this ion from the medium opens the junctional complex between most cells, including intestinal cells [38], oxyntic cells of gastric glands [39], pancreatic acinar cells [40], and monolayers of MDCK cells [41]. Switching cultured epithelial cells from growth in low (<5 μM) Ca^{++} to normal Ca^{++} levels induces junction assembly and formation of transepithelial electrical resistance, the so-called "Ca^{++}-switch" protocol [42,43]. Studying the process of tight-junction assembly and sealing in MDCK cells, Balda et al. [44] demonstrated that the activation of the phospholipase C/protein kinase C pathway enhances the development of transepithelial resistance. In contrast, inhibition of calmodulin blocks the development of transepithelial resistance. Placing cultured monolayers in Ca^{++}-free medium causes loss of ZO-1 from the membrane; switching these cells back into Ca^{++}-containing medium causes reassembly of ZO-1 on to the tight junction [31]. Preliminary experiments indicate that, even in the absence of calcium, the activation of protein kinase C induces the association of the ZO-1 protein with the junctional area [32]. Furthermore, if cells are treated with a protein kinase C inhibitor, ZO-1 does not associate with the membrane at the junctional area during the Ca^{++} switch [45]. Protein kinase C action is not simply involved in junction assembly, however, since the disassembly of tight junction induced by removal of extracellular Ca^{++} is blocked by PKC inhibitors [46]. These observations suggest that the PKC pathway may have direct effects on the protein components of the tight junction, altering their assembly and disassembly, but whether the Ca^{++}-switch model is applicable to physiologic regulation is unclear.

The Role of the Protein Kinase A Pathway

The role of the adenylyl cyclase-protein kinase A pathway has also been studied and implicated in junctional regulation. In some epithelia, the transepithelial resistance increases in response to increases in cAMP [47–49], in others it decreases [50–52], and, in one study, the increase in permeability of tight junctions induced by cAMP was selective for Cl^- [53]. There is evidence that the actions of protein kinase A and C are antagonistic. For example, in the Ca^{++}-switch model of junction assembly described above, protein kinase A activation inhibits junction assembly, while protein kinase C activation augments it. The lack of a consistent response and the failure of some studies to differentiate between changes in paracellular permeability from effects on membrane conductance make it difficult to define the site or mechanism of protein kinase A-mediated regulation.

The Role of the Membrane-Associated Cytoskeleton

Evidence for the cytoskeletal regulation hypothesis comes from diverse but complementary observations. The anatomic approximation of microfilaments to the tight-junctional region of epithelial cells suggests a functional relationship. Indeed, several investigators have observed that drugs active against microfilaments change tight-junctional permeability and/or structure in a variety of epithelia [42,54–56]. The observation that vasopressin stimulates myosin light-chain phosphorylation coincident with increased junctional permeability in the liver supports this idea. Several slow-acting hormones have been demonstrated to have coincident effects on actin organization and paracellular resistance. For example, the addition of insulin to established monolayers of human colonic epithelial cells (T84) causes the transepithelial resistance to decline more than eightfold over a three- to four-day period.

Together with this increased permeability, a subtle condensation of the perijunctional actin ring has been observed [57]. Interferon gamma appears to affect cultured intestinal epithelial cells in a similar fashion [58].

Recent studies performed in rodent intestine have demonstrated that glucose and amino acids can induce a significant increase in paracellular permeability coincident with condensation of the perijunctional microfilaments [59]. This discovery has led to the fascinating hypothesis that nutrient solutes trigger contraction of the membrane-associated cytoskeletal ring, increase junctional permeability, and enhance absorption through solvent drag [59–61]. Presumably the intermediary signal could be one of the second messenger cascades discussed above.

In summary, the regulation of tight-junction permeability seems to be a multi-step process which involves several signal transduction pathways, activated through different stimuli in different cell types. The loci for regulation are poorly defined but may involve both specific regulation of junction proteins or alterations in cytoskeletal organization.

PATHOLOGIC ALTERATIONS OF THE TIGHT JUNCTION

Alterations in tight junctions occur in a number of experimental and clinical conditions affecting the gastrointestinal system. These alterations, in general, have been observed either at the structural level, manifested by changes in the appearance of the junction, or at the functional level, manifested by changes in indirect measures of paracellular permeability. Many of the pathologic conditions such as common bile duct (CBD) obstruction of the liver [62,63] and many of the agents toxic to junctions, such as aspirin [64] and ethanol [65] in gastric mucosa, have complex effects on the tissues studied, and the mechanism of injury to the tight junction and the contribution of junction dysfunction to the observed pathology are difficult to determine. Nonetheless, analysis of some of these pathologic conditions and agents provides insight into junctional pathophysiology.

Phalloidin, an agent that stabilizes filamentous actin, when administered experimentally, decreases bile flow [46,66] and results in a marked accumulation of microfilaments around canaliculi in hepatocytes [67]. These overt cytoskeletal changes are accompanied by disorganization and decreased numbers of hepatocyte tight junction fibrils visualized by freeze-fracture electron microscopy and by increased junctional permeability to electron-dense tracers [66]. This increased junctional permeability is thought to contribute to cholestasis by dissipating electrochemical gradients across the canaliculus and allowing the reflux of bile from the canaliculus through the junction into the sinusoid. These observations imply that an intact apical cytoskeleton is required to maintain normal hepatocyte tight junction structure and function. The effects of two other microfilament toxins, cytochalasin D, which causes peri-junctional actomyosin ring contraction and increased tight junction permeability in the intestine [56], and cytochalasin B, which disperses microfilaments in hepatocytes and also impairs bile flow [68], support the notion that disruption of the cytoskeleton in turn disrupts the tight junction.

Interestingly, two inherited pediatric cholestatic liver disorders—Byler's disease [69] and North American Indian childhood cirrhosis [70]—have histologic features which are quite similar to experimental phalloidin injury and may be examples of inborn defects in cytoskeletal organization which result in tight-junction dysfunction.

Another form of cholestasis, that associated with CBD obstruction, also results in

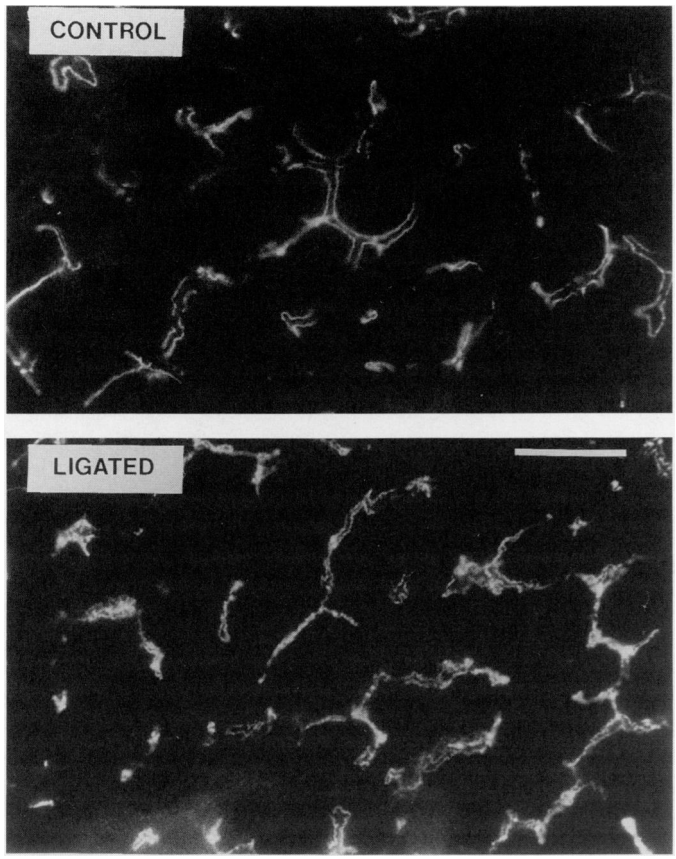

FIG. 2. Indirect immunofluorescence localization of ZO-1 in hepatocyte tight junctions of rat liver, before (control) and 72 hours (ligated) after ligation of the common bile duct. In normal liver, ZO-1 is positioned continuously along tight junctions which border both sides of the canalicular membrane surfaces. Following common bile duct ligation, the localization of ZO-1 becomes irregular and in places discontinuous. This change suggests loss of integrity of the structure which seals the paracellular space as a contributing factor in cholestasis.

structural and functional disruption of the tight junction. Electron microscope studies in experimental models [62], and in human tissue [63] have shown ultrastructural alterations similar to those seen with cytoskeletal disruption induced by phalloidin and have demonstrated increased paracellular permeability to electron-dense tracers. More recently, the immunolocalization of a tight junction-specific protein, ZO-1, has been shown to be disturbed after experimental CBD obstruction [71], supporting the theory that the molecular structure of the junction is disrupted in this form of injury (Fig. 2). Taken together, these findings support a role for tight junction disruption in the pathogenesis of obstructive cholestasis and suggest that primary injury to the cytoskeleton may underlie the disruption of the tight junction. It is tempting to speculate that similar cytoskeletal and tight junction damage may be important in the pathogenesis of injury related to obstruction of other luminal organs such as the bowel, the pancreas, and the kidney.

Intestinal epithelial tight junction abnormalities have also been observed in experimental and clinical pathologic conditions. Studies in a human intestinal epithelial cell line (T84) have shown that *Clostridium difficle* toxin A markedly decreases transepithelial resistance by increasing tight junction permeability. This permeability defect occurs in the absence of inflammatory cells, is associated with cytoskeletal disruption as measured by staining of the actin-binding drug rhodamine-

phalloidin, and is postulated to contribute to diarrhea by dissipating transepithelial electrochemical gradients needed for proper intestinal absorption and secretion [72]. Another junctional toxin designated ZOT has been found in attenuated *Vibrio cholerae* vaccine strains which do not produce cholera toxin but retain the ability to cause mild diarrhea. This toxin decreases ileal transepithelial resistance and alters the freeze-fracture electron microscopic appearance of the junction in a manner similar to phalloidin and extrahepatic biliary obstruction [73]. These observations again suggest that cytoskeletal damage may be a primary event in many forms of junction injury and suggest that toxin injury of the cytoskeleton and tight junction may be an under-appreciated mechanism of diarrhea production by infectious agents.

A variety of drugs and clinical agents have also been shown to disrupt intestinal epithelial tight junctions. For example, the effects of aspirin on junction structure when applied for short periods of time to canine gastric mucosa have been assessed [64]. Here, epithelial tight junction ultrastructure is distorted and junctional permeability to electron-dense tracers increased after exposures of less than one hour. Ethanol [65] and other agents (reviewed in [1]) also disrupt gastric mucosal junction ultrastructure and have led to the concept that many agents may initiate and amplify gastric mucosal injury by increasing tight junction permeability and allowing back-diffusion of H^+ ions into the mucosa.

The idea that increased intestinal tight junction permeability may increase the predisposition to mucosal damage by weakening the intestinal barrier is not confined to noxious agents in the stomach. There is ultrastructural [74] and functional evidence [75] that patients with Crohn's disease and their relatives have abnormally increased tight junction permeability. These observations suggest that in some patients with inflammatory bowel disease a primary defect which allows entrance of inflammatory agents into the mucosa is abnormal tight junction permeability [76].

Although our understanding of tight-junction pathophysiology is rudimentary, it is evident that epithelial tight-junction abnormalities occur after a wide variety of insults to the gastrointestinal system. A recurring theme is emerging that disruption of the cytoskeleton results in disruption of the tight junction. This pathophysiologic link is less surprising when the close structural relationship between the cytoskeleton and the tight junction is considered. As we understand more about the tight junction and the cytoskeleton and they are viewed less as static barrier and scaffolding and more as dynamic, closely interacting cellular components, then it may be less surprising that some pathologic junction states will be an extension of normal physiologic regulation of the tight junction.

CONCLUSION

The last 30 years have seen accelerated recognition that the properties of the paracellular path between epithelial cells is quite variable and physiologically regulated. Progress in understanding the molecular basis of these properties has only very recently come through identification and characterization of several protein components of the tight junction. Here we have suggested that the tight junction be considered a component of the apical cytoskeleton and that much physiologic regulation and pathologic alteration can be considered in terms of alteration in cytoskeletal, and actin, organization. Second-messenger signaling pathways (e.g., PKC, Ca^{++}) may affect the cytoskeleton or act directly on junction components through protein phosphokinases and phosphatases. Detailed insight into how the

paracellular barrier of epithelia is regulated and altered in disease will require more information about its molecular structure. The goal of this information is to define ways to correct pathologically defective barrier properties, prevent these defects, or to alter them for therapeutic purposes such as drug delivery.

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