The Renal Lesions of Alport Syndrome

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ABSTRACT

Alport syndrome is a hereditary, progressive, hematuric nephropathy characterized by glomerular basement membrane abnormalities with frequent hearing defects and ocular anomalies. The disease is associated with mutations in genes encoding the α3, α4, or α5 chains of type IV collagen, *COL4A3*, or *COL4A4* in the autosomal forms of the disease, *COL4A5* in the more frequent X-linked variety. Ultrastructural changes in the glomerular basement membrane and frequent abnormal expression of type IV collagen chains in renal and skin basement membranes are crucial elements for the diagnosis of Alport syndrome, determination of the mode of inheritance, and genetic counseling. Animal models have provided invaluable tools to study the mechanisms leading to progressive deterioration of the glomerular basement membrane and ultimately to renal failure, and to evaluate benefits of potential targeted therapies.

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CASE REPORT

A 10-yr-old boy was seen because of a 3 d episode of macroscopic hematuria occurring shortly after an upper respiratory tract infection. BP and a physical examination were normal. Blood cell count and serum creatinine level (65 μmol/L) were in the normal range. Urine analysis showed 100mg/d microalbuminuria and 10^6 red blood cells per mn on Addis count. Kidneys were normal by ultrasound. Discrete hearing loss was detected by audiometry.

His mother, a 37-yr-old female, was followed in the adult nephrology unit because of persistent microscopic hematuria discovered on routine urine analysis during a medical screen before employment. She never developed proteinuria or micro-albuminuria, and her GFR was normal. Her husband was said to be healthy.

Because of the possibility of Alport syndrome, a skin biopsy was performed in the boy. It was not informative, as the expression of the α5 chain of type IV collagen on the epidermal basement membrane (BM) was normal. On renal biopsy, podocyte hypertrophy was seen by light microscopy. No specific immune deposits were detected by immunofluorescence. However, using antibodies against type IV collagen chains, a complete lack of staining of the glomerular basement membrane (GBM) for the α3, α4, and α5(IV) chains was observed, contrasting with the normal α5(IV) labeling of Bowman capsule and collecting duct BMs. On ultrastructural examination, irregular alternation of thick and split, and of thin GBM segments was observed. These morphologic findings led to the diagnosis of autosomal recessive Alport syndrome.

Alport syndrome (AS) is a hereditary disease of the GBM characterized by the familial occurrence of progressive, hematuric nephropathy with sensorineural deafness. It accounts for 0.3 to 2.3% of all patients reaching end-stage renal disease (ESRD). The disease is genetically heterogeneous, as it is associated with mutations in one of the genes, *COL4A3*, *COL4A4* on chromosome 2, or *COL4A5* on chromosome X, encoding the α3(IV), α4(IV), and α5(IV) chains of type IV collagen. In the glomerulus, these three chains are synthesized by the podocyte.

The α(IV) chains have a long collagenous domain, with a glycine every third amino acid, and a noncollagenous domain at the C-terminal. They associate to form triple helical type IV collagen molecules that assemble into three different networks to constitute the backbones of BMs. The α1-α1-α2(IV) network is a component of all BMs whereas the α3-α4-α5(IV) network is present in the GBM, in the ear, eye, and lung BMs; and the α5-α5-α6(IV) in the skin and smooth muscle cell BMs.[1]

Whatever the gene mutated, macroscopic or microscopic hematuria, initially isolated, is the major sign of the disease. Other signs—proteinuria, increasing with age, renal failure and its rate of progression, and the presence and severity of extrarenal symptoms (deafness, ocular changes)—vary with the

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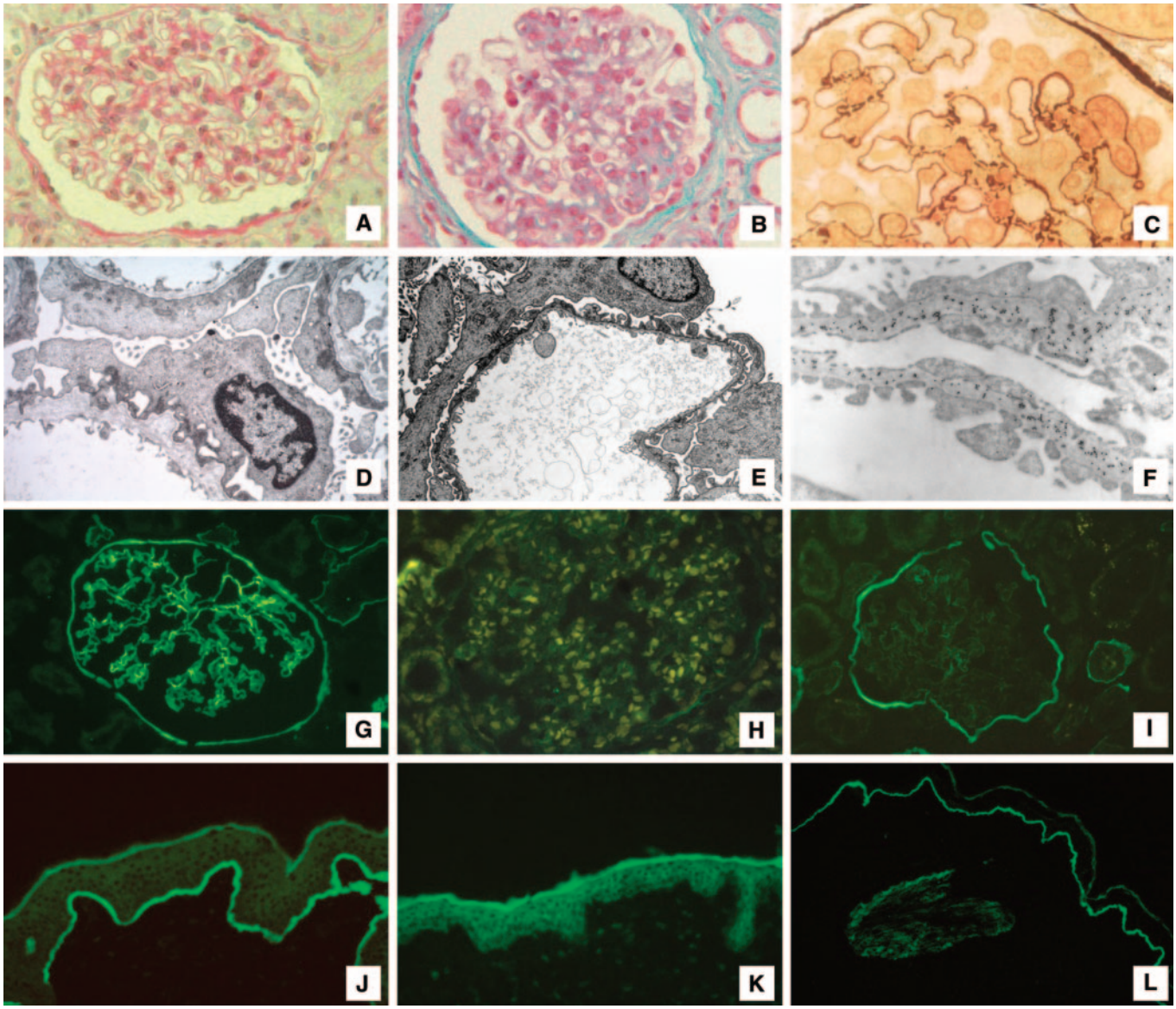

Figure 1. Alport syndrome. (A–C) Light microscopy. (A) Podocyte hypertrophy on an early biopsy specimen (Trichrome-light green x250). (B) Rigidity and moderate thickening of the glomerular basement membrane (GBM), and segmental glomerular sclerosis (Trichrome-light green x250). (C) Irregular thickening of the GBM (Silver methenamine x450). (D–F) Electron microscopy. (D) Irregular distribution of thick and split, and of thin GBM segments (Uranyl acetate-lead citrate x7000). (E) Diffusely thin GBM (Uranyl acetate- lead citrate x 11000). (F) Immunogold microscopy. Anti-α1(IV) chain antibody: Distribution of the gold particle within the full thickness of the GBM (x8000). (G–L) Immunofluorescence: Distribution of the α5(IV) chain in the kidney (G–I) and the skin (J–L) basement membranes. In control (G, J) the antibody stain the GBM, the capsular BM, the distal tubule BM and the epidermal BMs. In a male patient affected with X-linked Alport syndrome (H, K), no labeling is observed (there is a nonspecific diffuse fluorescence of the epidermis). In a patient affected with autosomal recessive Alport syndrome (I, L), the GBM is unstained but the capsular and collecting duct BMs are labeled as well as the epidermal BM.

type of mutation, and the sex of the affected patient in X-linked AS.

Renal Biopsy

In early biopsy specimens, renal tissue appears normal by light microscopy. However, podocyte hypertrophy (Figure 1A) and stiffness of the capillary wall (Figure 1B) are frequently observed, associated or not with the presence of tubular red blood cell casts. Then, focal and segmental thickening of the GBM may be seen on silver stains (Figure 1C), with progressive enlargement of the mesangial stalks. Development of segmental, then diffuse, glomerular sclerosis in an increasing number of glomeruli leads to complete sclero-hyalinosis of the tuft. Foci of tubulointerstitial lesions, which may precede clear-cut glomerular changes, increase in size and severity.

Clusters of interstitial foam cells are frequently found in proteinuric patients, and their number usually decreases in ESRD kidneys. No significant arterial changes are initially observed.

Immunofluorescence is initially negative; however, faint and irregular deposits of IgG, IgM, and/or C3 may be observed. More frequently, granular C3 deposits are irregularly distributed on the glomerular tuft and the afferent arterioles. With progression of the lesions, deposits of IgM, C1q, and C3 are seen in glomerular segmental lesions.

None of the previous anomalies are specific. Identification of ultrastructural changes of the GBM was thus of utmost importance as it provided a specific marker of the disease and suggested that AS is a disorder of the GBM. Typically, the renal lesion is characterized by diffuse thickening and splitting of the GBM with strikingly irregular outer and inner contours. But, most often, especially in young patients or in females with X-linked AS, irregular alternation of thick and abnormally thin GBM is observed (Figure 1D). Moreover, diffusely thin GBM, with smooth inner and outer contours, is the only GBM changes observed in 10 to 20% of patients, mostly in children, but also in adults with typical AS (Figure 1E).

X-Linked Alport Syndrome

X-linked AS accounts for about 85% of AS families. In males, the disease is severe. Proteinuria usually appears in childhood and increases steadily with age, with possible development of nephrotic syndrome, and constant progression to ESRD. Progressive hearing loss initially affecting high and middle frequencies, was observed in 79% of the male patients affected with X-linked AS in a large European cohort, and anterior lenticonus, a conic protrusion of the anterior aspect of the lens that is typical of AS, is seen in 13% of patients.[2] Asymptomatic perimacular yellow dots and flecks are specific markers of AS, and their occurrence is found at variable frequency (30 to 70%) by different groups.[3,4] Nonspecific corneal dystrophy and recurrent ulcerations have also been observed. In females, the severity of the nephropathy is quite variable. In the European cohort, 95% of female carriers have hematuria, 12% reach ESRD by the age of 40 and about 30% by the age of 60, discrete and delayed hearing loss is detected in 28% and asymptomatic maculopathy in 15%.[5]

Hundreds of *COL4A5* mutations have been identified, and nearly all of them are private. These include missense (frequently affecting glycines), nonsense, splicing mutations, as well as deletions and complex rearrangements. In males, large rearrangements, nonsense, and frameshift mutations confer higher risk of developing ESRD and deafness before 30 yr of age, and of having lenticonus, than missense *COL4A5* mutations.[2] Conversely, there is no phenotype-genotype correlation in females.[5] The rare association of AS with diffuse leiomyomatosis, a benign tumoral process involving smooth muscle cells of the esophagus, trachea and genital apparatus in females, and sometimes congenital cataract, is consistently linked to deletions removing the first two exons of the *COL4A6* gene located immediately 5′ to *COL4A5,* in the reverse orientation, and extending variably in *COL4A5*.[6]

Analysis of type IV collagen chain expression in kidney and skin basement membranes, using monoclonal antibodies, proves very useful in the diagnosis of X-linked AS. In about 70 to 80% of males, kidney examination shows a lack of α5(IV), α3(IV), and α4(IV) in the GBM (Figure 1H), and the distal tubular basement membrane where, normally, they are assembled (Figure 1G) in the same protomer, and of α5-α6(IV) in the Bowman and the collecting duct basement membranes. This abnormal distribution is associated with a strong expression of α1-α2(IV) throughout the width of the GBM (Figure 1F), whereas these chains are normally confined to the mesangium and the endothelial aspect of the GBM. In the skin biopsy (obtained by punch biopsy), the same patients show the co-absence of α5(IV) and α6(IV) chains in the epidermal basement membrane[7] (Figure 1K) where, normally, the α5-α5-α6(IV) network is present (Figure 1J), allowing a simple approach to diagnosis. In related females, the labeling—both in the skin and in the glomerulus—is usually segmental, due to random inactivation of the X chromosome. However, because of the patchy distribution, normal staining or complete lack of expression may be observed. In 20 to 30% of X-linked males and carrier females, the mutated chain assembles in the type IV collagen network, and the labeling—both in the kidney and the skin—is normal.[2] This normal expression usually is observed in a subset of missense mutations with late progression toward ESRD.

Autosomal Forms of AS

In the autosomal recessive form of AS, associated with homozygous or compound heterozygous mutations in either *COL4A3* or *COL4A4*, the disease is as severe in females as in males, and early progression toward ESRD—hearing defect, and retinopathy[4]—are frequent. Clinical and morphologic features are comparable to the X-linked form of AS. This mode of inheritance must be suspected in consanguineous families, when the disease is severe in a young female, and/or when the father of an affected male shows microhematuria. Sometimes X-linked inheritance can be ruled out by haplotype analysis (when two affected brothers have inherited a different *COL4A5* allele from their mother). Immunohistochemical study of skin and renal BMs may be normal, but shows, in most cases, abnormal pattern typical of this mode of inheritance: the GBM does not stain for α3-α4-α5 chains because of the lack of α3 or α4(IV), which precludes the assembly of the α3-α4-α5 protomer, whereas the Bowman capsule BM (Figure 1I) as well as the skin BM normally express the α5-α6(IV) network (Figure 1L).

The spectrum of phenotype in related individuals carrying heterozygous mutations is very wide. Some individuals are completely free of symptoms, whereas many others display intermittent or permanent microhematuria, associated with thin GBM in rare cases studied, without proteinuria and with stable renal

function. These latter features are typical of "familial benign hematuria." Rarely related individuals display an intermediate phenotype with hematuric disease with some level of proteinuria and/or renal failure.[8]

At the end of the spectrum, heterozygous *COL4A3* or *COL4A4* mutation is associated with autosomal dominant AS, which involves only a few families. In this form, male to male transmission can be observed, the renal disease progresses slowly in males as well as in females and, when documented, the electron microscopy shows thick, thick and thin, or diffusely thin GBM, and the hearing loss, when present, is a late event.[9] The expression of type IV collagen is normal in the few cases that have been studied.[9]

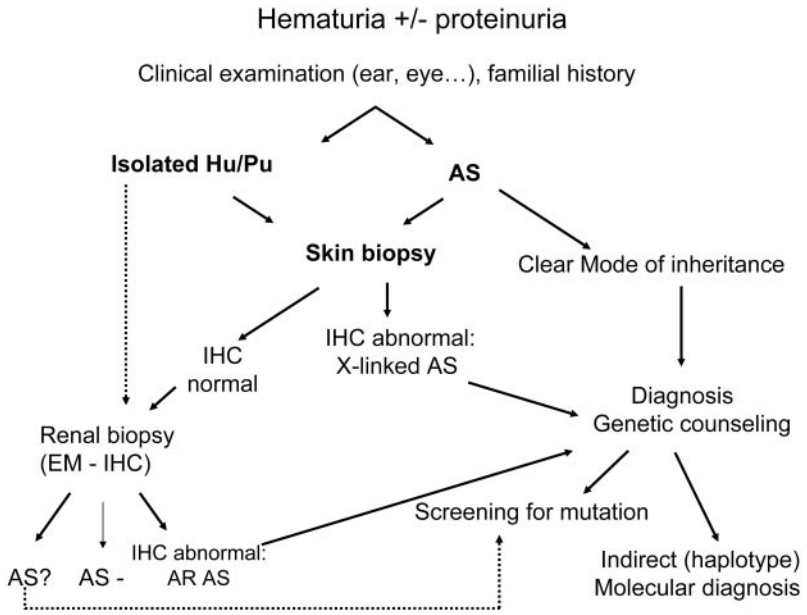

Figure 2. Schematic algorithm in case of Alport syndrome suspicion because of hematuria ± proteinuria.

Diagnosis of AS

The diagnosis is straightforward when clinical renal and extra-renal symptoms as well as family history are typical of AS. However, the lack of family history does not exclude the diagnosis of AS, as approximately 10% of *COL4A5* mutations occur *de novo*.[10] In this case, the presence of deafness should raise suspicion, and the finding of ocular abnormalities may lead to the diagnosis. Lack of α5(IV) expression in skin BM allows rapid and definitive diagnosis and genetic counseling, as it affirms X-linked transmission. However, the lack of extra-renal symptoms as well as the normal expression of the α5-α6 (IV) network does not rule out the diagnosis. In the presence of sporadic persistent hematuria, associated with proteinuria without any other symptoms suggesting AS, renal biopsy is frequently the first investigation performed. Examination of the renal tissue can exclude other hematuric glomerular disease, the most frequent being IgA nephropathy, and often shows typical ultrastructural abnormalities of the GBM when electron microscopy is performed, although exclusively thin BM can be observed. Glomerular hematuria, with proteinuria and deafness, can also be observed in *MYH9* related disease, with familial macrothrombocytopenia variably associated with glomerulopathy and deafness,[11] as well as in mitochondrial diseases.

The mode of inheritance of the disease (and therefore the genetic counseling) may be difficult to assess, and beyond cases of *COL4A5* neomutations, the genealogy sometimes does not allow definitive conclusions. The pattern of expression of type IV collagen chains, when abnormal, is very helpful to address this issue. However, normal distribution of type IV collagen chains in skin and kidney basement membranes may be observed in AS, and, in some families, the mode of inheritance cannot be definitively assessed. This is frequently the case when young children present with sporadic hematuria and diffusely thin GBM, which may correspond either to AS or familial benign hematuria, and in families with only females affected with microhematuria.

COL4A3, *COL4A4*, and *COL4A5* contain respectively 48, 52,and 53 exons. Direct sequencing of all coding exons from genomic DNA allows the finding of about 80% of mutations, but is laborious, time consuming, and expensive. Indirect diagnosis by haplotype analysis can be performed in informative families when expression of type IV collagen has established definitive diagnosis and mode of inheritance. Sequencing is currently performed for diagnostic purposes when the skin and kidney expression of the α(IV) chains is normal, and/or when there is an issue of genetic counseling. However, sequencing does not always provide a definitive diagnosis: for example, the finding of a single *COL4A3* or *COL4A4* mutation in a young child with normal α(IV) chains expression may correspond to a recessive form in which the second mutation was not found, to familial benign hematuria or to an autosomal dominant AS. Sometimes, only extended follow up will reveal the patient's phenotype. Figure 2 schematizes the algorithm that can be proposed for diagnosis of AS.

Pathophysiology and Therapeutic Approaches

The basic defect in Alport syndrome is either the lack, in the mature GBM, of the α3-α4-α5(IV) network and its failure to replace the α1-α2 network, which is known to be less resistant to proteolysis, or the presence of a defective α3-α4-α5(IV) network. There are several animal models for AS, in dogs and mice that faithfully recapitulate autosomal and X-linked forms of the disease. They have brought novel data to the understanding of the mechanisms responsible for the progression of AS nephropathy and in

the elaboration of future therapies. The re-expression of the α3(IV) chain in *Col4a3*$^{-/-}$ mice, for example, was shown to restore the expression of α4 and α5 (IV), thus demonstrating that the expression of all three α3-α4-α5(IV) chains is required for network assembly.[12] The downstream mechanisms responsible for progressive alteration of the GBM and renal failure are not fully understood. In young Alport mice, the ultrastructurally normal GBM is known to already be abnormally permeable.[13] The concomitant accumulation of mRNAs encoding TGFβ1 and extracellular matrix components in human and mouse Alport podocytes are thought to reflect key events in renal disease progression.[14] Blocking the TGFβ1 pathway prevents GBM thickening in Alport mice.[15]

The role of metalloproteinases in Alport disease has been underlined by recent studies. Increased expression of MMP2, MMP3, and MMP9 has been described, both at the transcriptional and the protein level, in AS kidneys in humans, mice, and dogs.[16,17] Such MMP up-regulation is not unique to Alport nephropathy. However, AS kidney basement membranes were shown to be more readily degradable *in vitro* by collagenase, elastase, and cathepsins, compared with normal kidney basement membranes,[18] and this is thought to be due to the lack of the highly cross linked α3-α4-α5(IV) network. Blocking simultaneously at least MMP2, MMP3, and MMP9 in *Col4a3*-/- mice delays the progression of the disease if treatment is given before development of GBM injury and occurrence of proteinuria in a C57BL6 genetic background.[16] In addition, a recent study found an increase of MMP12 expression in podocytes of humans, mice, or dogs affected with AS, possibly linked to MCP1-mediated activation of the podocyte CCR2 receptor.[19] Either MMP12 inhibitor or CCR2 receptor antagonist attenuates the GBM thickening in *Col4a3*-/- mice.[19]

Pharmacologic therapeutic approaches have been tested in animal models and in humans. Cyclosporine A was found to delay progression of renal failure in humans and dogs in initial studies.[20,21] However, cyclosporine is also found to be rapidly associated with nephrotoxicity, thereby precluding its long-term use.[22] Angiotensin-converting enzyme inhibitors and/or angiotensin 2 type 1 receptor antagonists reduce urinary protein excretion and preserve glomerular filtration in dogs affected with X-linked AS, in *Col4a3*-/- mice,[23] and in a few pediatric patients.[24] Larger controlled studies are necessary in humans to clarify the long-term benefit of the treatment and the nature and doses of drugs that are effective. Also, criteria for micro- or macroalbuminuria for starting renoprotective treatment by blockade of the renin-angiotensin system remain to be precisely determined. In Alport mice, chemokine receptor-1 blockade as well as statin treatment improves survival and renal lesions.[25] Finally, bone marrow transplantation of *Col4a3*-/- mice shows recruitment of bone marrow cells as future podocytes and mesangial cells, partial restoration of the expression of the α3-α4-α5(IV) network, and clinical and histologic improvement.[26–28] However, a recent study suggested that irradiation, which preceded bone marrow transplantation, may improve the survival of *Col4a3*-/- mice by itself, through as yet unidentified mechanisms.[29]

Overt anti-GBM nephritis occurs in only 3 to 5% of transplanted Alport patients.[30] The risk of graft loss is very high, and treatment with plasmapheresis and cyclophosphamide has shown limited benefit. The risk of recurrence on subsequent transplantation is very high. This complication is more likely to occur in patients with deletions or frameshift mutations, who do not express the α3α4α5(IV) GBM network. However, many patients with *COL4A5* deletion have been successfully transplanted, without developing anti GBM nephritis, and predictive factors for developing the disease are currently unknown.

DISCLOSURES

None.

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