Uptake and metabolism of biotin by human peripheral blood mononuclear cells

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Zempleni, Janos, and Donald M. Mock. Uptake and metabolism of biotin by human peripheral blood mononuclear cells. Am. J. Physiol. 275 (Cell Physiol. 44): C382–C388, 1998.—We studied the uptake of biotin into human peripheral blood mononuclear cells (PBMC) using [3H]biotin and studied the catabolism of biotin in PBMC using [14C]biotin. Over 30 min, [3H]biotin uptake was greater at 37°C than at 25°C (K1 = 2.6 ± 0.4 nM, maximal velocity = 2.9 ± 0.2 fmol · 10^6 cells⁻¹ · 30 min⁻¹). Ouabain reduced [3H]biotin uptake to 65% of control values, suggesting that biotin uptake is Na-K-ATPase dependent. Unlabeled biotin and biotin analogs reduced the uptake of [3H]biotin to 22–70% of control values, suggesting the presence of a competition for a structurally specific biotin transporter. When endocytosis by PBMC was stimulated by various acyl glycerols, [3H]biotin uptake was 40–73% of control values; these data are consistent with the hypothesis that stimulated endocytosis reduces biotin transporter density on the cell surface. During a 168-h incubation, PBMC did not catabolize [14C]biotin.

1,2-dioctanoyl-sn-glycero; endocytosis; sodium-potassium-adenosinetriphosphatase

BIOTIN HOMEOSTASIS IN MAMMALS appears to be affected by pregnancy and by drug treatment. For example, the catabolism of biotin to inactive metabolites is accelerated in pregnancy (17, 23, 24) and during therapy with aniconvulsants (13, 15, 19, 22). In these situations, the mechanisms leading to the disturbances in biotin homeostasis have not yet been fully explored. For in vitro studies, this gap in knowledge is due to a lack of appropriate model systems in which to study the metabolism of biotin in humans.

For mechanistic studies at the cellular level, characterization of a readily available human cell line should promote research in this area. In this paper, we describe studies of human peripheral blood mononuclear cells (PBMC) as a model system for studies of biotin metabolism in human cells. We characterized 1) the uptake kinetics of biotin into PBMC, 2) the specificity and mechanism of biotin uptake into PBMC, and 3) biotin catabolism in PBMC.

MATERIALS AND METHODS

Isolation of PBMC. Human peripheral blood was collected in heparinized syringes (12 units of heparin/ml blood). PBMC were isolated by gradient centrifugation using Histopaque-1077 (Sigma Chemical Co., St. Louis, MO); the PBMC were washed twice using Dulbecco’s PBS without magnesium chloride or calcium chloride (Sigma). The PBMC were resuspended in PBS at ~7.6 × 10^6 cells/ml. Cell viability was measured by the ability to exclude 0.16 mM trypan blue. Mean ± SD viability of freshly isolated PBMC was 96 ± 3% and did not change significantly during the experiments with [3H]biotin. In the 3-h incubations with [14C]biotin, PBMC viability was determined at each time point of cell harvesting; viability was 99.5% at the beginning and 97.8% after 168 h.

When PBMC are isolated by the gradient centrifugation procedure as described above, one obtains a cell preparation that essentially contains only lymphocytes. In blood, lymphocytes and monocytes are quantitatively the most important mononuclear cells; eosinophils and basophils account for ~3% of total PBMC (26). The monocytes are removed during the process of cell isolation because of their selective binding to plastic surfaces.

Uptake experiments using [3H]biotin. In a typical experiment, 900 µl of PBMC suspension were mixed with 5 µl of [3H]biotin. When the effects of cations on [3H]biotin uptake were studied, sodium phosphate in the incubation media was replaced by equimolar potassium phosphate, and sodium chloride in the media was replaced by either choline chloride, lithium chloride, or ammonium chloride. PBMC were washed in these media before the incubations. To investigate the effects of inhibitors and stimulators of biotin uptake, the aliquot of PBS was replaced by various solutions of compounds dissolved in PBS plus additional solubilizers as specified below. The PBMC were preincubated at 37°C with 2.4-dinitrophenol or iodoacetate (10 min), ethionine or ouabain (20 min), various acyl glycerols (30 min), or colchicine (60 min). In all other experiments, the PBMC were warmed at the designated temperature for 5 min before addition of [3H]biotin, unlabeled biotin, or biotin analogs. When PBMC were warmed in control medium for either 5 min or as long as 60 min, no significant difference in [3H]biotin uptake was seen (data not shown). Unlabeled biotin, biotin analogs, metabolic inhibitors, and acyl glycerols were purchased from Sigma.

After preincubation of the PBMC suspensions, 5 µl of [3H]biotin solution (sp act 2.15 TBq/mmol; Dupont, Boston, MA) were added to the suspensions. The final biotin concentration in the assays was 244 ± 61 pM; the serum concentration of biotin plus metabolites is 464 ± 178 pM (20). The temperatures and durations of the subsequent incubations are specified in results.

After incubation, PBMC were sedimented from the incubation mixture by centrifugation at 2,260 g for 100 s. The supernatant was discarded, and the PBMC pellet was resuspended in PBS. After washing three times to remove extracellular [3H]biotin, the PBMC pellet was dissolved in a small volume of PBS and was transferred into a scintillation vial containing 0.5 ml of 0.5% Triton X-100. The incubation vial was washed two times; these washings were added to the scintillation vial, and liquid scintillation fluid (4.5 ml of Ultima Gold XR; Packard Instruments, Meriden, CT) was added. [3H] was quantitated in a liquid scintillation analyzer Tri-Carb 1900-TR (Packard). Blanks were prepared by mixing PBMC suspension at 4°C with [3H]biotin and by washing
the cells immediately. Radioactivity in blanks was <3% of the radioactivity measured for the incubated samples; similar values were measured when blanks were prepared by the omission of PBMC. True biotin uptake was calculated as the difference between the values measured for the incubated samples and that measured for the blanks. All assays were done in triplicate.

Some of the compounds used in the incubations are poorly water soluble. The concentration of α,γ-lipoic acid (Sigma) in solution was verified by measuring the absorbance at 330 nm, using ε = 150 M⁻¹·cm⁻¹ (31). Bilirubin (Sigma) was dissolved in 2 ml DMSO and was further diluted using PBS; the bilirubin concentration was verified by measuring the absorbance at 453 nm (ε = 60.7 M⁻¹·cm⁻¹) (36). The final concentration of DMSO in the incubation experiments using bilirubin was <2%. 1,2-Dioctanoyl-sn-glycerol, 1,3-dioctanoyl-glycerol, 1-monocaprylyl-rac-glycerol, and 1,2-didecanoyl-rac-glycerol were dissolved individually in DMSO and were further diluted with four volumes of PBS to give acyl glycerol concentrations at 3.16 mM; the final concentration of DMSO in the incubation experiments using these acyl glycerols was 1%.

Endocytic uptake of sucrose. Sucrose is a commonly used marker to measure fluid phase endocytosis by cells (7, 9). It is an appropriate substrate to measure endocytosis by PBMC because PBMC do not express sucrase activity (10). Because some of our experiments dealt with the effects of endocytic activity in PBMC on biotin uptake, we quantitated the endocytic uptake of [¹⁴C]sucrose in our studies with stimulators of endocytosis. PBMC were incubated with [¹⁴C]sucrose (Amersham; sp act 22.8 GBq/mmol) at 15.9 kBq/ml cell suspension. The cells were incubated for 30 min at 37°C as described above for the uptake of [³H]biotin. Results are expressed as endocytic index (µl of fluid endocytosed·10⁶ cells⁻¹·30 min⁻¹). The endocytic index equals a true volume of endocytosis only if the marker compound that is analyzed does not bind to the cell surface, which appears to be true for sucrose (25).

Incubation experiments using [¹⁴C]biotin. The radiolabel of [³H]biotin is released from the molecule during the catabolism of biotin by β-oxidation. In contrast, the radiolabel stays on the biotin metabolite if [¹⁴C]carboxyl-labeled biotin is β-oxidized (16). Therefore, we used [¹⁴C]carboxyl-labeled biotin to study the catabolism of biotin by PBMC. Freshly isolated PBMC were suspended aseptically in RPMI 1640 medium with L-glutamine and HEPES (Atlanta Biologicals, Norcross, GA). Ten milliliters of autologous plasma, 10,000 units of penicillin, and 10 mg streptomycin were mixed with 90 ml of the medium. [¹⁴C]biotin was added to give a final concentration at 482 nM biotin (Amersham; sp act 2.0 GBq/mmol). The relatively small specific radioactivity of [¹⁴C]biotin compared with [³H]biotin made it necessary to use greater biotin concentrations compared with the experiments with [³H]biotin.

For incubations, PBMC were added to produce a final concentration of 5.51 × 10⁶ cells/ml and were incubated at 37°C in a 48-well plate. The gas composition in the incubator was held constant at 5% carbon dioxide-95% oxygen. PBMC suspensions and controls were harvested at 0 h (before incubation) and 24, 48, 72, 96, and 168 h after onset of incubation. As a control for chemical (nonbiological) degradation of biotin, we incubated [¹⁴C]biotin without PBMC.

The samples were hydrolyzed with hydrochloric acid as described previously (21). After adjustment to pH 2.5 and filtration, [¹⁴C]biotin and [¹⁴C]biotin metabolites were separated by HPLC and were quantitated by radiometric flow detection as described previously (35). All assays were done in triplicate.

Statistics. Significance of differences among the treatment groups was tested by one-way ANOVA. Dunnett's post hoc procedure was used for post hoc testing; the Dunnett procedure compares the means as measured for the treatment groups to the control mean (1). SuperANOVA 1.11 (Abacus Concepts, Berkeley, CA) was used for the calculations. Differences were considered significant if P < 0.05.

RESULTS

Time course and temperature dependency. The biotin uptake into PBMC was linear with time over 30 min (Fig. 1). The uptake of biotin was less at 25°C than at 37°C (Fig. 1). All further incubations were made at 37°C. We investigated whether biotin uptake was also linear with time at the greatest biotin concentration as used in our uptake experiments; when PBMC were incubated at 37°C with 4,748 pM [³H]biotin, biotin uptake was linear with time (y = 68.2x + 346.9, r = 0.973).

Rates of uptake. The PBMC were incubated at various biotin concentrations for 30 min at 37°C, and the biotin uptake was measured (n = 4 subjects). At a physiological biotin concentration of 475 pM in the medium, the rate of biotin uptake was 450 ± 34 nmol·10⁶ cells⁻¹·30 min⁻¹ (Fig. 2A). Biotin uptake exhibited saturation with respect to the biotin concentration in the media. Lineweaver-Burk transformation of the data (Fig. 2B) produced a linear plot with Kₗ = 2.6 ± 0.4 nM and maximum velocity = 2.9 ± 0.2 fmol·10⁶ cells⁻¹·30 min⁻¹. Kₗ is the concentration of biotin required to half-saturate the rate of maximal transport under the experimental conditions.

Specificity. The uptake of [³H]biotin into PBMC was not affected by structurally similar compounds other than by unlabeled biotin or biocytin. When unlabeled biotin or biocytin was added at concentrations 8,200 times the concentration of [³H]biotin (3.9 µM vs. 475 pM), the uptake of [³H]biotin was 22.2 ± 3.8% (unlabeled biotin) and 33.1 ± 5.6% (unlabeled biocytin), respectively, of the [³H]biotin uptake without these competitors (Fig. 3A).
The following compounds with structural similarities to biotin were included in the specificity experiments: lipoic acid, which has a valeric acid side chain and a sulfur-containing ring like biotin; bilirubin, which has carboxyl groups, carbonyl groups, and imino groups like biotin; hexanoic acid, which has a carboxyl group and a similar chain length like the side chain in the biotin molecule. When the PBMC were incubated at a lipoic acid concentration similar to human plasma (0.05 µM) or at pharmacological concentrations (5 µM), the uptake of biotin was not different from controls. When the PBMC were incubated with bilirubin at concentrations as present in human plasma (4.8 µM), the uptake of biotin was not different from that in controls. Hexanoic acid at 5.0 µM did not affect the biotin uptake into PBMC.

We sought to determine whether biotin analogs in which the ring structure of the molecule has been modified can compete with [3H]biotin for uptake into PBMC. PBMC were incubated with [3H]biotin (475 pM) and either unlabeled biotin, D,L-desthiobiotin, 2-iminobiotin, or diaminobiotin at concentrations (3.9 µM) 8,200 times the concentration of [3H]biotin. In D,L-desthiobiotin the thiophane portion of the biotin ring is modified; in 2-iminobiotin and diaminobiotin the ureido portion of the biotin ring is modified. When PBMC were incubated with [3H]biotin and either unlabeled biotin, 2-iminobiotin, or diaminobiotin, the uptake of [3H]biotin was <30% of the [3H]biotin uptake without these competitors (Fig. 3B). When PBMC were incubated with [3H]biotin and D,L-desthiobiotin, the uptake of [3H]biotin was ~70% of the [3H]biotin uptake without this competitor.

Transport mechanism. PBMC were incubated with metabolic inhibitors or stimulators to characterize the biotin transport mechanism. When PBMC were incubated with ouabain, an inhibitor of Na-K-ATPase, biotin uptake was reduced to 65 ± 9.9% of the control value (Fig. 4). When PBMC were incubated with ethionine, a compound that lowers intracellular ATP concentrations, biotin uptake was not significantly different from controls. When PBMC were incubated with colchicine, a compound that disassembles microtubules, biotin uptake was not different from controls.

We sought to investigate whether the uptake of [3H]biotin into PBMC depends on the availability of sodium in the incubation medium. When PBMC were incubated in media where sodium was replaced by either choline, lithium, or ammonium, biotin uptake was reduced significantly to 16–48% of the control value (Fig. 5).

We sought to determine whether decoupling of the respiratory chain and modification of sulfhydryl groups...
affect the uptake of biotin into PBMC. We chose 2,4-dinitrophenol to decouple the respiratory chain and we chose iodoacetate to modify sulfhydryl groups (8); both compounds were added to give a final concentration of 1 mM (29). When PBMC were incubated with 2,4-dinitrophenol, biotin uptake was reduced to 50.65.9% of the control value (n = 6; P < 0.01). When PBMC were incubated with iodoacetate, biotin uptake was reduced to 73.66.7% of the control value (n = 6; P < 0.01).

We sought to determine whether changes in the rate of endocytosis affect the uptake of biotin into PBMC. Initially, we examined a dose-response curve for 1,2-dioctanoyl-sn-glycerol. This well-established enhancer of fluid phase endocytosis in PBMC and other cells (12) reduced biotin uptake linearly up to a concentration of 158 µM (Fig. 6). Thereafter, the inhibition reaches a plateau. Based on this finding, 1,2-dioctanoyl-sn-glycerol was used at a concentration of 158 µM for the further experiments.

We sought to determine whether 1,2-dioctanoyl-sn-glycerol affects the biotin uptake in PBMC from four subjects. We also studied whether effects of 1,2-dioctanoyl-sn-glycerol are specific for this compound or if such effects can also be caused by other acyl glycerols, namely 1,3-dioctanoylglycerol, 1-monocapryloyl-rac-glycerol, or 1,2-didecanoyl-rac-glycerol at equimolar concentrations. In these molecules, either the position of the acyl residues is different from that in 1,2-dioctanoyl-sn-glycerol, the number of acyl residues in the molecules is different, the chain length of the acyl residues is different, or a combination of these differences is present. When PBMC were incubated with 1,2-dioctanoyl-sn-glycerol, the biotin uptake was 61.13% of the control values (Fig. 7), reproducing the reduction observed in the dose-response experiments. The other acyl glycerols also reduced the biotin uptake by PBMC. Biotin uptake was reduced to 40–73% of control values in the presence of 1,3-dioctanoylglycerol, 1-monocapryloyl-rac-glycerol, or 1,2-didecanoyl-rac-glycerol.

DMSO was used to dissolve the various acyl glycerols (0.7–1.0% DMSO in the PBMC suspensions); as a control for the effect of DMSO per se, we examined DMSO at concentrations up to 1.9%. No significant reductions of biotin uptake were seen (data not shown).
As a control, we sought to verify that 1,2-dioctanoyl-sn-glycerol was truly increasing the endocytic activity in PBMC despite the observed reductions in biotin uptake. We measured the uptake of \(^{14}C\) sucrose, a commonly used marker to quantitate endocytosis (7, 9). The endocytic index of \(^{14}C\) sucrose was 0.040 ± 0.019 µl · 10^6 cells^-1·30 min^-1 in the control experiments. When PBMC were incubated with 1,2-dioctanoyl-sn-glycerol, the endocytic index of \(^{14}C\) sucrose increased to 0.072 ± 0.017 µl · 10^6 cells^-1·30 min^-1 (P < 0.05 vs. controls).

Metabolite profile. \(^{14}C\) carbonyl-labeled biotin was added to sterile PBMC preparations; aliquots of the PBMC suspensions and controls (without PBMC) were analyzed after 0, 24, 48, 72, 96, and 168 h of incubation. The PBMC did not catabolize the biotin. Intact biotin accounted for ≥97% of the radioactivity in each sample (data not shown). Biotin-d, l-sulfoxide accounted for the remainder, in an amount equal to that present in the zero-time sample and in the control incubation. Biotin-d, l-sulfoxide in the zero-time sample results from an impurity in the commercial biotin preparation. Biotin-d, l-sulfoxide is formed by sulfoxidation in the thioephane ring of the biotin molecule; this racemic mixture is present in commercially available biotin preparations at 1–5%.

**DISCUSSION**

The data of this study provide evidence that biotin uptake into human PBMC is mediated by a structurally specific, energy-dependent, and sodium-coupled transporter. This conclusion is based on the following findings: 1) biotin uptake was temperature dependent; 2) biotin uptake showed saturation kinetics; 3) an excess of unlabeled biotin or biotin analogs decreased the uptake of \(^3H\) biotin; 4) ouabain, 2,4-dinitrophenol, and iodoacetate decreased the uptake of biotin; 5) biotin uptake was decreased when extracellular sodium was replaced by other cations. Furthermore, these findings suggest that what we measured as biotin uptake was truly intracellular biotin rather than biotin nonspecifically adsorbed to cell membranes.

The \(K_T\) that was measured for biotin transport into PBMC is in the same order of magnitude as normal serum concentrations of biotin plus metabolites, i.e., ~1 nM (20). The \(K_T\) for biotin in PBMC is smaller than that reported for the biotin transporter in human liver basolateral vesicles at ~1 μM (27). The liver is exposed to great postprandial biotin concentrations in the portal vein compared with the biotin concentrations in peripheral circulation (2). Thus the larger biotin concentrations of portal vein blood could theoretically make the greater \(K_T\) for liver cells useful regulating biotin uptake. The rate of biotin uptake into hepatocytes (24.9 fmol · 10^6 cells^-1·min^-1) is ~1,700 times greater than that measured for PBMC in the present study (3). Clearly, hepatocytes are capable of absorbing a greater total mass of biotin than PBMC.

The biotin transporter in PBMC appears to be structurally specific for biotin and biotin metabolites. An excess of either unlabeled biotin or biotin analogs significantly reduced the uptake of \(^3H\) biotin, but organic acids that are less structurally similar to biotin did not affect biotin uptake. These findings distinguish the biotin transporter in PBMC from biotin transporters in other cells. Bowers-Komro and McCormick (3) observed that the uptake of \(^3H\) biotin (20 nM) into rat hepatocytes in the presence of 20 μM bilirubin was 37% of the control values. In the human hepatoma cell line Hep G2, unlabeled biotin or lipoic acid at concentrations 6,250 times the concentration of \(^3H\) biotin (at 4 nM) reduced biotin uptake by at least 50%; in contrast, the addition of unlabeled biocytin had no effect (28). In rat intestine, various biotin analogs at concentrations 50 times the concentration of \(^3H\) biotin (at 1 μM) reduced biotin transport by at least 60% (29).

Our data suggest that the thioephane portion in the biotin molecule plays an important role for binding to the biotin transporter in PBMC. When biotin was modified at the carboxyl group (biocytin) or at the ureido portion of the ring system (2-iminobiotin, diaminobiotin), these compounds competed with \(^3H\) biotin for uptake into PBMC to a similar extent as unlabeled biotin. In contrast, when biotin was modified at the thioephane portion of the ring system (d, l-desethylbiotin), this compound decreased the uptake of \(^3H\) biotin less effectively.

Based on our observation of decreased biotin uptake in the presence of ouabain, biotin uptake into PBMC is dependent on normal function of Na-K-ATPase. It seems likely that biotin is cotransported with sodium, because biotin uptake into PBMC was reduced when extracellular sodium was replaced by choline, lithium, or ammonium. Likewise, as observed by others, the biotin uptake into rat hepatocytes, rat intestinal cells, or Hep G2 cells was reduced by ouabain and by sodium-free media (3, 28, 29). Consistent with a sodium-driven transport is the effect of the decoupler 2,4-dinitrophenol; decoupling of the respiratory chain reduces the generation of ATP, thereby reducing the activity of Na-K-ATPase and thereby reducing transcellular sodium gradients. We would also have expected to see ethionine affecting biotin uptake into PBMC, since the effects of ethionine are thought to be mediated by reductions in intracellular ATP concentrations (3). However, biotin uptake was not reduced in the presence of ethionine. We speculate that ethionine did not decrease intracellular ATP concentrations in the present study sufficiently to impair biotin transport.

Colchicine did not affect biotin uptake into PBMC in these studies. In contrast, colchicine has been shown to inhibit the transport of protein (avidin)-bound biotin by adsorptive pinocytosis into HeLa cells and human fibroblasts (4–6). Our studies did not reproduce the published results showing adsorptive pinocytosis in HeLa cells and fibroblasts. We conclude that endocytosis is not an important mechanism for biotin transport into PBMC.
Nevertheless, the rate of endocytosis has a striking impact on biotin transport into PBMC. When fluid phase endocytosis was stimulated by 1,2-dioctanoyl-sn-glycerol, biotin uptake was reduced to 61% of control values. The effect is not due to a reduction of endocytosis per se; the endocytosis of [14C]Sucrose increased by 97% in response to 1,2-dioctanoyl-sn-glycerol. Stimulation of [14C]Sucrose uptake reported here is reasonable. At concentrations of 1,2-dioctanoyl-sn-glycerol similar to the one used in the present study, Keller et al. (12) reported a 20% increase in endocytosis of FITC-dextran by PBMC.

Structurally similar acyl glycerols reduced biotin uptake by a percent similar to 1,2-dioctanoyl-sn-glycerol. This result is consistent with the report that 1-oleyl-2-acetylglycerol and 1,2-dicaprylglycerol stimulated fluid phase endocytosis in PBMC and neutrophils (11, 12).

To explain decreased biotin uptake in response to increased endocytosis, we hypothesize that the density of biotin transporters on the cell surface is transiently reduced by endocytosis. Removal of proteins and receptors from the cell surface during endocytosis is a well-established event. Concanavalin A receptors are removed from pig epidermal cell surface at a reduced rate in response to colchicine or cytochalasin B (34). The proportion of interiorized membrane can be large; for example, ~50% of the cell surface area can be interiorized during each hour of endocytic activity in fibroblasts (33). Although endocytosed membrane receptors (probably including transporters) can be recycled to the cell membrane within minutes (33), endocytosed biotin transporters may not belong to this class of recycled receptors or the process of recycling may have not been completed during the course of our incubations. It has been proposed that endocytosed membrane receptors may be replenished from an intracellular pool (33). Perhaps no such pool exists for the biotin transporters on PBMC membranes.

Our 168-h incubations with [14C]Biotin provide evidence that PBMC do not catabolize biotin in vitro. Similarly, Said et al. (28) found that the human hepatoma cell line Hep G2 did not catabolize [3H]biotin; ~94% of the intracellular radioactivity was associated with biotin. We conclude that both the [3H] and the [14C] detected in the present studies represent biotin rather than radiolabeled metabolites.

Are PBMC an appropriate model to study biotin metabolism in human cells? We conclude that PBMC are useful to study situations that affect the transport of biotin into cells, e.g., hormonal effects in pregnancy or changes in the rate of membrane turnover and hence the turnover of biotin transporters on the cell surface. PBMC are less useful in predicting whether there will be competition between biotin and structurally similar compounds for uptake into other cells, e.g., hepatic transport (3, 27, 28). This may limit the use of PBMC in studies of the effect of anticonvulsant therapy on biotin metabolism, especially if this interaction arises from transport competition (14, 18, 30).

Our studies provide evidence that PBMC are not useful to study the cellular catabolism of biotin. Unfortunately, the liver is quantitatively much more important than PBMC with regard to biotin uptake (27), storage (32), and catabolism (16). Human PBMC are not a good model for studies on biotin catabolism despite the ease of access.

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REFERENCES