Elevated glutamine metabolism in splenocytes from spontaneously diabetic BB rats

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To investigate the metabolic fates of glutamine in splenocytes from the BB rat with spontaneous immunologically mediated insulin-dependent diabetes, freshly isolated cells were incubated in Krebs–Ringer Hepes buffer with 1.0 mM- $[U-^{14}C]$ glutamine and 0, 4 mM- or 15 mM-glucose. (1) The major products of glutamine metabolism in splenocytes from normal and diabetic rats were ammonia, glutamate, aspartate and CO_2 . (2) The addition of glucose increased ($P < 0.01$) glutamate production, but decreased ($P < 0.01$) aspartate and CO_2 production from glutamine, as compared with the values obtained in the absence of glucose. However, there were no differences in these metabolites of glutamine at 4 mM- and 15 mM-glucose. (3) At all glucose concentrations used, the productions of ammonia, glutamate, aspartate and CO_2 from glutamine were all markedly increased ($P < 0.01$) in splenocytes from diabetic rats. (4) Potential ATP production from glutamine in the splenocytes was similar to that from glucose, and was increased in cells from the diabetic rat. (5) ATP concentrations were increased ($P < 0.01$) in diabetic-rat splenocytes in the presence of glutamine with or without glucose. (6) Our results demonstrate that glutamine is an important energy substrate for splenocytes and suggest that the increased glutamine metabolism may be associated with the activation of certain subsets of splenocytes in the immunologically mediated diabetic syndrome.

INTRODUCTION

Glutamine is the most abundant amino acid in the plasma of animals and humans [1]. It serves as an important precursor for the synthesis of important molecules such as purine and pyrimidine nucleotides, NAD^+ and amino sugars, which participate in a variety of biochemical processes [1]. Therefore it is not surprising that glutamine is essential for the division of cells in culture [2] and that the utilization and metabolism of glutamine are extensive in dividing cells such as enterocytes, fibroblasts and certain tumour cells [3].

Previous studies have demonstrated that lymphocytes have high activities of both glutaminase and the enzymes of the tricarboxylic acid cycle [4,5]. Furthermore, these cells show high rates of glutamine metabolism, with glutamate, ammonia and aspartate as the major products [6–14]. This incomplete glutamine metabolism has been termed ‘glutaminolysis’, by analogy to the glycolysis of glucose [8,15]. The rate of glutamine utilization by lymphocytes increases in response to stimulation by mitogenic agents [6–14]. Thus it has been suggested that glutamine plays an important role in the function of lymphocytes and immune responses [8,16–18]. However, we are unaware of prior studies of glutamine metabolism in cells of the immune system in conditions that are associated with impaired immune function and/or autoimmunity.

The spontaneously diabetic BB rat is a widely studied animal model of immunologically mediated insulin-dependent diabetes mellitus that shares many characteristics with human type I diabetes [19–23]. The BB-rat diabetes is believed to be an autoimmune disease in which activated lymphocytes (and perhaps macrophages) specifically infiltrate the pancreatic islets and destroy the insulin-producing β -cells. The syndrome is also characterized by immunological defects, including the presence of autoantibodies, T-cell lymphopenia, defective allograft rejection, and marked decrease in the proliferative response *in vitro*

to mitogens by lymphocytes from blood or peripheral lymphoid organs [20–23]. This decreased proliferative response by these cells may be due to an abnormality in the binding of the antigen or mitogen to the cell membrane receptors, or in signal transduction, but does not necessarily suggest decreased cellular activity. Indeed, we have recently demonstrated an increase in the cellular metabolism of glucose in freshly isolated splenocytes from diabetic [24] and diabetes-prone [25] BB rats. These results are consistent with other data suggesting activation of certain cells of the immune system in diabetic BB rats [22,26]. Furthermore, an innate abnormality of energy metabolism in such cells cannot be excluded [24,25]. In view of the essential role of glutamine for cells of the immune system [16–18], the present study was carried out to characterize glutamine metabolism in splenocytes from diabetic BB (BBd) rats, and to compare it with that of control non-diabetes-prone (BBn) rats. To distinguish the effects of the diabetic state itself from the effects caused by immunological alterations, glutamine metabolism was also studied in splenocytes from BBn rats with chemically induced diabetes.

MATERIALS AND METHODS

Chemicals

$[U-^{14}C]$ Glutamine was obtained from Amersham Corp., Oakville, Ont., Canada. Its radiochemical purity was determined to be over 99% by t.l.c. as previously described [27]. L-Lactate dehydrogenase, glutamate dehydrogenase, α -oxoglutaric acid, ATP, D-luciferin and luciferase were obtained from Boehringer Mannheim, Montreal, Que., Canada. Hyamine hydroxide, BSA (fraction V), streptozotocin and other chemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Animals

Male and female BBd rats and age- and sex-matched control

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BBn rats were obtained from Dr. Pierre Thibert (Animal Resources Division, Health Protection Branch, Ottawa, Ont., Canada). Animals were housed in a light (12 h-light/12 h-dark cycles)- and climate-controlled facility in laminar flow hoods and provided with laboratory rat chow (Ralston-Purina, Woodstock, Ont., Canada) *ad libitum* and water. Diabetic rats (15–30 days post-onset) were maintained on single daily subcutaneous injections of ultralente insulin (2–5 units) [24].

Chemically (streptozotocin) induced diabetes in BBn rats

Male BBn rats (90 days old) were injected intravenously with streptozotocin (65 mg/kg body wt.) in 50 mM-sodium citrate (pH 4.5). Rats became diabetic within 24 h of the injection, as indicated by hyperglycaemia measured with Chemstrip.bG (Boehringer Mannheim, Canada) and by glycosuria measured with Chemstrip.uG (Boehringer Mannheim). Diabetic rats were maintained on single daily subcutaneous injections of ultralente insulin (2 units) for 4 days before use for metabolic studies.

Preparation of splenocytes

Rats (115–140 days old) were anaesthetized with ether. Spleens were removed and passed through a 50-mesh grid in cold Krebs–Ringer Hepes buffer (pH 7.4) [28] supplemented with 0.5% BSA. After centrifugation at 200 *g* for 10 min at 4 °C, red blood cells were lysed for 3 min in a solution consisting of 155 mM-NH₄Cl, 0.1 mM-disodium EDTA and 10 mM-KHCO₃. Splenocytes were washed three times and then suspended in the buffer described above. Cell viability was determined by Trypan Blue exclusion, and results are presented per 10⁶ viable cells.

Incubation of splenocytes

Triplicate incubations were performed at 37 °C in 10 ml silicone-treated tubes. Freshly prepared splenocytes were incubated for 2 h at a concentration of 5 × 10⁶ cells/ml, in a total volume of 1 ml of the Krebs–Ringer Hepes buffer (pH 7.4) [28] supplemented with 0.5% BSA, 1 mM-[U-¹⁴C]glutamine (150 d.p.m./nmol) and glucose (0, 4 or 15 mM) as indicated in the Tables. The glutamine concentration of 1 mM was chosen for use in this study because it is close to that in normal rat plasma, namely 0.7 mM, as measured by h.p.l.c. [29]. Tubes were gassed with 100% O₂ for 20 s. Incubation was initiated by the addition of cells and terminated by the addition of 100 µl of 1.5 M-HClO₄ to the incubation medium through the stopper. At the end of the incubation period, ¹⁴CO₂ was collected in 200 µl of Hyamine hydroxide, which was added through the stopper into a 500 µl micro-centrifuge cup suspended in the tube. Shaking was continued for 1 h to trap the ¹⁴CO₂ evolved. Radioactivity in ¹⁴CO₂ was measured with a LKB 1217 Rackbeta counter (Pharmacia, Baie d'Urfe, Que., Canada) by using a modified Bray's solution [30] as previously described [31]. In preliminary experiments, it was established that ¹⁴CO₂ production and metabolite formation from glutamine were linear during a 2 h incubation period. Parallel incubations, in which incubation medium was acidified with 100 µl of 1.5 M-HClO₄ before addition of cells, were included as blanks. Blank radioactivity was subtracted from sample radioactivity.

Measurement of lactate, pyruvate, ammonia, [¹⁴C]glutamate, [¹⁴C]aspartate and amino acids

Lactate and pyruvate in acid extracts of cells plus medium were determined by automated fluorimetric enzymic methods [32]. Ammonia in neutralized samples was measured by a spectrophotometric enzymic method [33]. [¹⁴C]Glutamate and [¹⁴C]-aspartate produced from [¹⁴C]glutamine were separated on Dowex AG 1-X8 (200–400 mesh, acetate form) (Bio-Rad Labor-

atories, Mississauga, Ont., Canada) as previously described [34]. Their radioactivities were measured in the liquid-scintillation counter with Universal Cocktail (ICN Radiochemicals, Montreal, Canada). Individual free amino acids in acid extracts of cells plus medium were analysed by h.p.l.c. as previously described [29].

Potential production of ATP from glutamine

This was calculated based on the following assumptions: 9 mol of ATP for 1 mol of net aspartate produced [17] and 24 mol of ATP for 5 mol of CO₂ production via the complete oxidation of glutamine [35], which was equal to total CO₂ production minus aspartate production, as stoichiometrically 1 mol of CO₂ is produced for 1 mol of aspartate formed [13].

Measurement of ATP in splenocytes

Splenocytes were incubated as described above. At the end of the 2 h incubation period, ATP was extracted from the cells by heating the samples in a boiling-water bath for 5 min as previously described [36]. After cooling in ice, the samples were centrifuged for 15 s in a Micro-centrifuge (Fisher Scientific, Montreal, Canada). A 50 µl portion of the supernatant was immediately used for determination of ATP using luciferase as previously described [37], except that the photons were measured by liquid-scintillation spectrometry [38] rather than with a luminometer [37].

Statistical analyses

Results were statistically analysed by the one-way analysis of variance with SNK multiple comparison test, paired or unpaired *t* tests [39] as indicated in Tables.

RESULTS AND DISCUSSION

Glutamine metabolism in splenocytes from BBn rats

Glutamine was metabolized mainly to glutamate and ammonia, and somewhat less to aspartate and CO₂ in BBn splenocytes in both the presence and the absence of glucose (Table 1), indicating partial metabolism of glutamine in these cells. Our results are consistent with the previous findings on the metabolic fates of glutamine reported for splenocytes, thymocytes, peripheral blood lymphocytes and mesenteric lymphocytes [6–14]. In the absence of glucose, only a small amount of pyruvate and no detectable lactate was formed in the presence of glutamine as the sole substrate (Table 1). There was no significant amount of alanine, asparagine or amino acids other than glutamate and aspartate formed in the splenocytes from glutamine in the medium [40]. It is noteworthy that the rate of ¹⁴CO₂ production from [U-¹⁴C]glutamine in splenocytes, which was similar to that recently reported for resting splenocytes [11], was about 30 times that reported for mesenteric lymphocytes [17]. This difference could not be explained by the higher activities of glutaminase (a rate-limiting enzyme in glutamine metabolism) in mesenteric-lymph-node lymphocytes than in splenocytes [41]. It could, however, be due to the different tissues of origin for the lymphocyte preparations and/or the use of high cell density (5 × 10⁷ cells/ml) [17]. In support of the latter suggestion, we have shown that the metabolism of glucose [24] and glutamine [42] per 10⁶ splenocytes was markedly decreased as cell concentrations in the incubation medium were increased to 100 × 10⁶ cells/ml.

Glutamine metabolism in splenocytes from BBd rats

The metabolic patterns of glutamine in splenocytes from BBd rats in the presence and absence of glucose were qualitatively similar to those found for BBn rats (Table 1). These data suggest

Table 1. Glutamine metabolism in splenocytes from BBn and BBd rats

Freshly isolated splenocytes were incubated for 2 h in the presence of 1 mM-[U-¹⁴C]glutamine with or without the addition of glucose as described in the text. Values are expressed as means $\pm$ S.E.M. The number of animals for each treatment is indicated in parentheses. The means with different superscripts within groups of BBn and BBd rats are significantly different ($P < 0.01$) as analysed by one-way analysis of variance or paired t test. All mean values for BBd rats are significantly higher ($P < 0.01$) than the corresponding values for BBn rats as analysed by the unpaired t test. Abbreviation: ND, not detectable.

Product	Glucose concn. ...	Product formation (nmol/2 h per 10 ⁶ cells)					
		BBn rats			BBd rats		
		0	4 mM	15 mM	0	4 mM	15 mM
¹⁴ CO ₂ (12)		12.7 $\pm$ 1.7 ^a	8.7 $\pm$ 0.7 ^b	9.2 $\pm$ 0.8 ^b	24.8 $\pm$ 3.1 ^a	18.4 $\pm$ 1.3 ^b	16.9 $\pm$ 1.1 ^b
[¹⁴ C]Glutamate (8)		5.3 $\pm$ 0.5 ^a	6.8 $\pm$ 0.4 ^b	7.1 $\pm$ 0.7 ^b	9.1 $\pm$ 0.8 ^a	11.2 $\pm$ 0.6 ^b	12.7 $\pm$ 0.9 ^b
[¹⁴ C]Aspartate (8)		3.2 $\pm$ 0.3 ^a	1.8 $\pm$ 0.1 ^b	1.9 $\pm$ 0.1 ^b	5.9 $\pm$ 0.6 ^a	2.8 $\pm$ 0.3 ^b	2.6 $\pm$ 0.2 ^b
Ammonia (8)		13.0 $\pm$ 1.4	13.7 $\pm$ 1.0	15.1 $\pm$ 1.6	26.2 $\pm$ 3.1	23.3 $\pm$ 1.7	24.3 $\pm$ 1.5
Pyruvate (10)		0.4 $\pm$ 0.1 ^a	3.8 $\pm$ 0.2 ^b	4.9 $\pm$ 0.3 ^c	0.9 $\pm$ 0.1 ^a	5.8 $\pm$ 0.4 ^b	7.2 $\pm$ 0.4 ^c
Lactate (10)		ND	14.4 $\pm$ 0.9 ^a	22.9 $\pm$ 2.2 ^b	ND	25.5 $\pm$ 1.3 ^a	31.0 $\pm$ 2.8 ^b

the absence of intrinsic defects in the pathways involved in glutamine metabolism in BBd-rat splenocytes. However, the rates of production of glutamate, aspartate, ammonia and CO₂ from glutamine were all markedly increased ($P < 0.01$) in BBd-rat splenocytes at all glucose concentrations used (Table 1), indicating an increase in glutamine metabolism. These results are consistent with the hypothesis of prior activation *in vivo* of certain subset(s) in splenocytes from BBd rats [22–26]. These results also suggest that the increased glutamine metabolism in BBd splenocytes is not likely to be due to hyperglycaemia in the diabetic donor, but rather it is associated with immunological alterations. This suggestion is further supported by our findings that glutamine metabolism in the presence and absence of glucose was not increased in splenocytes from chemically (streptozotocin) induced diabetic BBn rats (Table 2).

Splenocytes, like thymocytes and mesenteric lymphocytes, are heterogeneous and contain different cell populations (as defined functionally and by monoclonal antibody labelling) including B-cells, helper T-cells, cytotoxic/suppressor T-cells, natural killer cells and macrophages [40]. At present, there is no information in the literature on substrate metabolism in the purified subsets of lymphocytes and macrophages from spleen, although mouse peritoneal macrophages have been reported to be extremely active in utilizing glutamine and glucose [35]. Owing to the heterogeneity of cells within the splenocyte population, and

because the make-up of this population differs between normal and diabetic animals [40], it is not clear which cells in the splenocyte preparations are associated with the increased glutamine metabolism. Further studies are required to investigate whether the enhanced glutamine metabolism in BBd splenocytes is due to altered numbers of cells of specific subsets, activation of one or more lymphocyte subsets and/or different interactions between and among subsets.

Effects of glucose on glutamine metabolism in splenocytes from BBn and BBd rats

There are differing reports in the literature as to the effect of glucose on lymphocyte glutamine metabolism. Ardawi & News-holme [6] first showed that glucose increased the production of both glutamate and aspartate in mesenteric lymphocytes from normal rats. Subsequently, Ardawi [14] reported that glucose also increased ¹⁴CO₂ production from [U-¹⁴C]glutamine by peripheral blood lymphocytes. In contrast, Brand *et al.* [7,9] demonstrated that glucose decreased the production of glutamate, aspartate and ¹⁴CO₂ from [U-¹⁴C]glutamine by thymocytes. These qualitative differences have been suggested to be due to the differing functional maturity of the lymphocytes studied [9,11].

In the present study, 4 mM- and 15 mM-glucose increased ($P < 0.01$) [¹⁴C]glutamate production from [¹⁴C]glutamine in splenocytes of both BBn and BBd rats as compared with the values

Table 2. Glutamine metabolism in splenocytes from control BBn and streptozotocin-induced diabetic BBn rats

Freshly isolated splenocytes were incubated for 2 h in the presence of 1 mM-[U-¹⁴C]glutamine with or without the addition of glucose as described in the text. Values are expressed as means $\pm$ S.E.M. ($n = 6$). The means with different superscripts within groups of BBn and diabetic BBn rats are significantly different ($P < 0.05$) as analysed by one-way analysis of variance. There were no differences in any of the measured end products of glutamine metabolism between control BBn and streptozotocin-induced diabetic BBn rats.

Product	Glucose concn. ...	Product formation (nmol/2 h per 10 ⁶ cells)					
		BBn rats			Streptozotocin-induced diabetic BBn rats		
		0	4 mM	15 mM	0	4 mM	15 mM
¹⁴ CO ₂		11.4 $\pm$ 0.9 ^a	8.8 $\pm$ 0.6 ^b	8.4 $\pm$ 0.5 ^b	12.2 $\pm$ 0.05 ^a	9.1 $\pm$ 0.4 ^b	9.2 $\pm$ 0.6 ^b
[¹⁴ C]Glutamate		5.1 $\pm$ 0.2 ^a	5.9 $\pm$ 0.3 ^b	6.0 $\pm$ 0.2 ^b	4.9 $\pm$ 0.2 ^a	5.8 $\pm$ 0.2 ^b	5.8 $\pm$ 0.1 ^b
[¹⁴ C]Aspartate		2.9 $\pm$ 0.3 ^a	1.7 $\pm$ 0.2 ^b	1.6 $\pm$ 0.2 ^b	3.0 $\pm$ 0.2 ^a	1.8 $\pm$ 0.2 ^b	1.6 $\pm$ 0.1 ^b
Ammonia		12.2 $\pm$ 0.8	13.5 $\pm$ 0.9	12.5 $\pm$ 1.2	12.6 $\pm$ 0.7	11.7 $\pm$ 0.8	11.9 $\pm$ 0.7

Table 3. H.p.l.c. analysis of glutamate and aspartate in cell extracts plus incubation medium of splenocytes

See Table 1 for experimental details. Glutamate and aspartate were analysed by h.p.l.c. Data are expressed as means $\pm$ S.E.M. ($n = 8$). The means with different superscripts within a row are significantly different ($P < 0.01$) as analysed by one-way analysis of variance or paired t test. All mean values for BBd rats are significantly higher ($P < 0.001$) than those for BBn rats as analysed by the unpaired t test. Abbreviation: ND, not detectable.

Amino acid	Type of rat	Product formation (nmol/2 h per 10^6 cells)		
		No glutamine	1 mM-Glutamine	
		+ 4 mM-glucose	No glucose	+ 4 mM-glucose
Glutamate	BBn	ND	5.72 ± 0.37^a	7.41 ± 0.32^b
	BBd	ND	9.86 ± 0.54^a	12.35 ± 0.74^b
Aspartate	BBn	1.70 ± 0.13^a	5.57 ± 0.32^b	3.34 ± 0.14^c
	BBd	2.66 ± 0.17^a	9.35 ± 0.57^b	5.29 ± 0.35^c

obtained in the absence of glucose (Table 1). Similar results were obtained from the measurement of glutamate by h.p.l.c. (Table 3), suggesting that the increased [^{14}C]glutamate production was unlikely to be due to radiolabel dilution. On the other hand, 4 mM- and 15 mM-glucose markedly decreased ($P < 0.01$) [^{14}C]aspartate production from [U- ^{14}C]glutamine in BBn and BBd splenocytes, as compared with the values obtained in the absence of glucose (Table 1). The inhibition of [^{14}C]aspartate production by glucose was also unlikely to be due to radiolabel dilution, since similar results were obtained from h.p.l.c. analysis of aspartate (Table 3). Our results are consistent with those of Spencer *et al.* [43], who found that glucose markedly decreased aspartate production from glutamine in a rat chondrosarcoma. We suggest that glucose metabolism augments the formation of citrate from glutamine-derived oxaloacetate, thus decreasing the availability of oxaloacetate for glutamate-oxaloacetate transamination. This may result in an increase in glutamate accumulation, with a concomitant decrease in aspartate production (Tables 1 and 3).

There were no differences in glutamine metabolism in splenocytes in the presence of 15 mM-glucose as compared with 4 mM-glucose (Table 1). This suggests that glutamine metabolism in lymphocytes may not be influenced by elevated glucose concentrations beyond the physiological range to values frequently occurring in diabetes. It also implies a substantial 'basal' utilization of glutamine by lymphocytes, particularly in BBd rats,

and further suggests an important role for glutamine as a substrate for these cells.

Potential production of ATP from glutamine in BBn- and BBd-rat splenocytes

In the absence of glucose, the partial metabolism of glutamine to aspartate could produce similar amounts of ATP to that from the complete oxidation of glutamine carbon via the tricarboxylic acid cycle in both BBn and BBd rats (Table 4). However, in the presence of 4 mM- and 15 mM-glucose, less ATP was produced from the partial metabolism of glutamine to aspartate without influencing the amount of ATP production from the complete oxidation of glutamine carbons via the tricarboxylic acid cycle (Table 4). As a result, the presence of 4 mM- and 15 mM-glucose only slightly decreased ($P < 0.05$) overall ATP production from glutamine as compared with the values obtained in the absence of glucose (Table 4). These results are consistent with previous studies suggesting that both glutamine and glucose can serve as substrates for lymphocytes [7–15,42]. It is noteworthy that there were no differences in potential ATP production from glutamine in the presence of 4 mM-glucose as compared with 15 mM-glucose (Table 4). This finding suggests that the elevated glucose concentrations in diabetes may not decrease the contribution of glutamine to lymphocyte ATP production. The increased glutamine metabolism observed in BBd-rat splenocytes resulted in an almost 2-fold increase in potential ATP production from glutamine at all glucose concentrations used (Table 4).

In the presence of 1 mM-glutamine, $^{14}\text{CO}_2$ production from 4 mM-[U- ^{14}C]glucose in splenocytes was 3.5 ± 0.2 in BBn ($n = 21$) versus 4.9 ± 0.9 in BBd ($n = 13$) (nmol/2 h per 10^6 cells; $P < 0.05$) (G. Wu, C. J. Field & E. B. Marliss, unpublished work). On the basis of this and lactate production (Table 1), ATP production from glucose was estimated [35] to be 36.7 and 56.5 nmol/2 h per 10^6 cells respectively in BBn and BBd splenocytes in the presence of both glutamine and glucose. Thus, of potential ATP production in the presence of 1 mM-glutamine plus 4 mM-glucose, glutamine could account for 56% and 61%, respectively, in BBn and BBd splenocytes. This further supports the concept that glutamine is an important energy source for splenocytes [42], in contrast with the recent conclusion by O'Rourke & Rider [11] that glutamine makes only a minor contribution to splenocyte ATP generation.

ATP concentrations in splenocytes from BBn and BBd rats

Small amounts of ATP were present in BBn-rat splenocytes at the end of a 2 h incubation period in the absence of glutamine or glucose (Table 5). However, much larger amounts of ATP were found in splenocytes from both BBn and BBd rats incubated in

Table 4. Potential production of ATP from glutamine in splenocytes from BBn and BBd rats

Production of ATP from glutamine is calculated as described in the text. Data are expressed as means $\pm$ S.E.M. ($n = 8$). The means with different superscripts within groups of BBn and BBd rats are significantly different ($P < 0.05$) as analysed by one-way analysis of variance. Means indicated by * are significantly different ($P < 0.01$) from the values obtained from the metabolism of glutamine to aspartate as analysed by the paired t test. All mean values for BBd rats are significantly higher ($P < 0.01$) than the corresponding values for BBn rats as analysed by the unpaired t test.

Source of ATP	Glucose concn. ...	ATP (nmol/2 h per 10^6 cells)					
		BBn rats			BBd rats		
		0	4 mM	15 mM	0	4 mM	15 mM
Gln to Asp		29.6 ± 3.2^a	17.0 ± 1.0^b	16.8 ± 0.9^b	53.8 ± 5.1^a	26.3 ± 1.4^b	23.0 ± 1.7^b
Gln to CO_2		35.1 ± 2.9	$30.2 \pm 2.5^*$	$35.3 \pm 3.5^*$	59.6 ± 1.5	$60.3 \pm 2.7^*$	$68.8 \pm 5.2^*$
Total		64.7 ± 3.8^a	47.3 ± 3.2^b	52.0 ± 4.1^b	113.5 ± 6.9^a	86.6 ± 5.4^b	91.7 ± 5.9^b

Table 5. ATP concentrations in splenocytes at the end of the 2 h incubation period

ATP in splenocytes was measured as described in the text. Data are given as means $\pm$ S.E.M., with numbers of animals indicated in the parentheses. The means with different superscripts within a row are significantly different ($P < 0.01$) as analysed by one-way analysis of variance. Means indicated by * are significantly ($P < 0.01$) different from the corresponding BBn group, as analysed by the unpaired t test.

Type of rat	Glucose...	No addition of substrates	ATP (pmol/ 10^6 cells)		
			1 mM-Glutamine		
			0	4 mM	15 mM
BBn (6)		19.0 $\pm$ 2.7 ^a	72.1 $\pm$ 9.4 ^b	89.7 $\pm$ 11.6 ^b	103.5 $\pm$ 11.6 ^b
BBd (7)		50.7 $\pm$ 4.9 ^{*a}	208.9 $\pm$ 12.5 ^{*b}	220.2 $\pm$ 28.7 ^{*b}	221.8 $\pm$ 19.1 ^{*b}

the presence of 1 mM-glutamine alone, which were not significantly increased by the addition of 4 mM- and 15 mM-glucose. These results further suggest the appreciable potential of glutamine to contribute ATP to splenocytes. ATP concentrations were markedly increased ($P < 0.01$) in splenocytes from BBd rats at all glucose concentrations used (Table 5). These results are consistent with our data on both glutamine metabolism (Table 1) and its potential contribution to ATP production (Table 4), which were all markedly increased in splenocytes from BBd rats.

Possible role of elevated glutamine metabolism in pathophysiology of immunologically mediated diabetes

We have previously reported that plasma concentrations of glutamine are increased 2–4-fold in the diabetic BB rat [19], probably due, at least in part, to increased muscle protein degradation [44]. The increase in plasma glutamine concentration may be necessary to satisfy the increased demand for glutamine by cells of the immune system, as we have demonstrated in the present study that glutamine metabolism is markedly enhanced in BBd splenocytes (Table 1).

The activation of the cells of the immune system, proposed to be involved in the process of destruction of the pancreatic β -cells [22], would require increased energy metabolism. The present study has shown, in addition to the increase in glutamine metabolism, the increased production of lactate and pyruvate from glucose in BBd-rat splenocytes (Table 1), as previously found [24,25]. In view of the suggestion that glutamine is an important substrate for lymphocytes, as presented herein and elsewhere [6,42], an increase in the metabolism of glutamine as well as of glucose may play an important role in providing cells of the immune system with both energy and the precursors for biosynthetic processes. In this regard, the increased glutamine metabolism as observed in BBd splenocytes may be associated with the activation of certain subset(s) of splenocytes in the immunologically mediated diabetic syndrome. On the other hand, increased ATP production from glutamine (Table 4) may be involved in the destruction of target cells by lymphocytes, as it has recently been suggested that ATP may act as a possible mediator of cell-mediated cytotoxicity [45]. It can be speculated that specific inhibition of or interference with glutamine metabolism in cells of the immune system may prevent activation of these cells and thus the development of diabetes. This could be analogous to the use of glutamine analogues to inhibit the growth of tumour cells [46] that also use glutamine as an important energy substrate and a biosynthetic precursor [3].

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