Increased tissue protein synthesis during spontaneous inflammatory bowel disease in HLA-B27 rats

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ABSTRACT

Inflammatory bowel diseases (IBDs) are associated with an increased whole-body protein turnover. In certain drug-induced experimental models of IBD, disturbances of protein synthesis in tissues have been reported recently, but it is unclear if similar disturbances occur in other chronic intestinal diseases. Therefore we investigated changes in protein synthesis in different tissues of HLA-B27 (human leucocyte antigen B27) transgenic rats that develop spontaneously chronic inflammation, with major involvement of the colon. Protein synthesis rate in HLA-B27 rats was shown to be higher in nine different tissues compared with control (Fisher 344) rats. The absolute rate of protein synthesis was highly stimulated at the main inflammatory site (+290% in the colon). However, liver, muscle and skin appeared to be major contributors to the increased protein synthesis observed at the whole-body level. Despite the increased protein synthesis, HLA-B27 rats presented a marked atrophy of muscles, which suggests an increased proteolysis. These results contrast with metabolic disturbances described in acute inflammation and colitis induced by drugs (i.e. dextran sodium sulphate). The present study suggests that the modifications of protein metabolism are strongly influenced by the type of the inflammatory diseases and thus by the underlying mechanisms, which result in different metabolic adaptations and specific nutritional requirements.

INTRODUCTION

Inflammatory bowel diseases (IBDs) involve inflammatory lesions of the intestine, especially in its distal part [1]. Weight loss associated with these chronic inflammatory diseases particularly affects the lean body mass [2]. This is reflected by a muscular loss, which can lead to a reduction of mobility and autonomy. A better knowledge of the protein metabolism disturbances occurring during these pathological states is needed to understand the mechanisms leading to muscle loss and how to manage these diseases more efficiently.

The metabolic disturbances that accompany acute injury are well known both at the whole-body and tissue levels. Whatever the causes, the disturbances of protein metabolism associated with acute inflammation...
have many similarities that suggest a common pattern of response, which differs mainly in duration and intensity according to the type of injury [3].

In contrast, only a few studies in humans have been devoted to protein metabolism in chronic diseases such as IBD. At the whole-body level, an increase of protein turnover has been described in IBD [4]. The increase in protein synthesis reported in the colon and liver of IBD patients [5] does not appear sufficient to explain the increase at the whole-body level [4].

The effects of IBD on protein metabolism in organs could be more extensively studied in animals, but this would require the choice of pertinent animal models. In experimental animal models, IBD can be induced by the injection of exogenous agents, for instance chemical substances such as 2,4,6-trinitrobenzene sulphonamide [6], indomethacin [7] or dextran sodium sulphate (DSS) [8]. In these cases, the onset of the disease is associated with an early acute inflammatory state with metabolic disturbances probably similar to those described in acute inflammation. In DSS-treated rats, a chronic inflammatory state can be maintained [9]. During this chronic period of inflammation, protein synthesis was recently found to be increased in organs of the splanchnic area, mainly in the spleen and the last part of the intestine. In the muscle, protein synthesis was decreased and, probably, proteolysis as well [9]. However, it is not clear if different chronic inflammatory pathologies of the intestine result in similar disturbances of protein metabolism. Genetic factors have a major influence in the development of IBD [10]. Recently, genetically modified animals spontaneously developing a chronic inflammatory disease were generated, such as HLA-B27 (human leucocyte antigen B27) transgenic rats [11]. The development of the inflammatory disease was progressive, persistent, and there were similarities to B27-associated human diseases, which commonly involve the gastrointestinal tract. HLA-B27 rats of the 33-3 line developed inflammatory lesions in the gut, joints, skin and in male genital organs [11], but the predominant manifestation occurred in the distal part of the gut [12,13].

The aim of the present study was to assess the effects of this spontaneous inflammatory disease on protein metabolism in different organs and to determine if the disturbances were similar to those observed in humans and other experimental models of IBD.

**MATERIALS AND METHODS**

**Animals and experimental design**

All procedures were performed according to current legislation on animal experimentation in France. Male HLA-B27/β2-microglobulin transgenic rats of the 33-3 line aged 8-weeks (n = 8; Iffa Credo; Saint Germain sur l’Arbresle, France) were housed in a temperature-controlled room (22–23 °C) in individual wire-bottom cages with a 12 h/12 h light/dark cycle. After a 10-day acclimatization period, food intake and body weight were measured twice a week until the end of the experiment (25-weeks old). Throughout the study period, rats had free access to water and to a semi-synthetic diet supplying the following: 650 g/kg of carbohydrate, 150 g/kg of protein (balanced to meet all amino acid requirements), 80 g/kg of lipids, 30 g/kg of crude fibre and minerals plus vitamins sufficient to maintain adults rats, as detailed previously [9]. All rats had free access to the same diet until 23-weeks old. As food intake was 11 % less in HLA-B27 rats than in controls in the 19- to 23-week old period (see Results), we decided to give the control rats the same amount of food as that consumed by the HLA-B27 rats until the end of the experiment. Thus pair-feeding conditions were performed during the last 2 weeks of the experiment to make sure that the modifications observed in HLA-B27 rats resulted from the inflammation and not from the difference in food intake. A blood sample was taken from a tail vein to measure the levels of acute-phase proteins at various times during the experimental period. At the end of the experimental period, the protein synthesis rate was estimated in different tissues according to the flooding-dose method [14]. In the postprandial state, animals received a bolus injection of L-[1-13C]valine (150 µmol/100 g of body weight; 99 % mole percentage excess, 0.5 ml/100 g of body weight) in a lateral tail vein. After 3 min, 200 µl of blood was sampled from a lateral tail vein for estimation of the decline of free [13C]valine enrichment between injection and death. Animals were anaesthetized with sodium pentobarbital (6 mg/100 g of body weight) and killed by blood puncture in the abdominal aorta 20 min after the valine injection. An aliquot was used for cell enumeration. Part of the sampled blood was collected on EDTA, centrifuged and the plasma was collected for analysis of L-[1-13C]valine enrichment, protein carboxyls, acute-phase proteins, i.e. fibrinogen, α1-acid glycoprotein (AGP), α2-macroglobulin (A2M) and albumin. The remaining blood was collected on heparin for blood glutathione assay. After the rats were killed, liver, spleen, lung, thymus, kidneys and muscles (gastrocnemius, soleus and extensor digitorum longus) were quickly removed, washed in PBS (pH 7.0) when necessary, blotted, weighed and frozen in liquid nitrogen. The tibialis anterior muscle was fixed in Bouin’s solution for histological analysis. The gut was isolated and washed with PBS. Each part of the gut (duodenum, jejunum, ileum and colon) was then cut into several pieces, frozen in liquid nitrogen and stored at −80 °C until analysis. Segments of the colon and ileum were also fixed in Bouin’s solution for histological analysis. An additional five Fisher 344 rats were killed without injection of L-[1-13C]valine for determination of
natural enrichment of protein-bound [1-\textsuperscript{13}C]valine in the different tissues studied.

**Blood parameters**

Plasma protein content was assayed using the Biuret method (Roche Diagnostic, Neuilly-sur-Seine, France). Blood cell counts were performed using an automatic analyser (Cobas Minos Vet; ABX, Montpellier, France). Acute-phase proteins were assayed by following systemic inflammation. Fibrinogen concentration was measured by turbidimetric method with a commercial kit (Biodyne; Les Ulis, France). The assays of AGP, A2M and albumin were performed by the single radial immunodiffusion method [15] using an anti-(rat albumin) antibody (ICN; Orsay, France) and anti-(rat AGP) and anti-(rat A2M) antibodies produced as described previously [16].

**Gastrointestinal inflammation**

Intestinal segments fixed in the Bouin’s solution were dehydrated with alcohol and embedded in paraffin. Histological sections of 5 µm were stained with haematoxylin and eosin for evaluation of inflammatory lesions. Cytokine mRNA levels were estimated by performing an RNA extraction and a specific amplification by reverse transcription–PCR. Total RNA from a tissue fragment (80–100 mg) powdered in liquid nitrogen was isolated using the acid phenol method [17] with a Micro RNA isolation kit (Stratagene, Amsterdam, The Netherlands). For each sample, cDNA was synthesized from 1 µg of total RNA by reverse transcription in a total volume of 20 µl, and cDNA of interest was amplified by PCR. The GeneAmp Gold RNA PCR kit was used for both steps (Applied Biosystems, Foster City, CA, U.S.A.). PCR amplification was performed using 1.5 µl of the reverse transcription product and specific primers for rat β-actin (forward 5′-TACAGCTTACACCACACAGC-3′, and reverse 5′-AAGGGAAGGCTGGAAAGAGC-3′, giving rise to a 206 bp product) and for the rat cytokines tumour necrosis factor α (TNFα), interleukin (IL)-6, IL-1α, IL-10 as described by Rivera et al. [18]. Results were visualized under UV light on a 2 % (w/v) agarose gel stained with ethidium bromide, photographed with a numeric camera and evaluated with ImageQuant™ 1.1 software (Molecular Dynamics, Orsay, France).

**Oxidative status**

Plasma and extensor digitorum longus protein carbonyl content (an index of oxidative stress) was measured as described previously [9]. Oxidized (GSSG) and reduced (GSH) glutathione levels were assayed in the blood, liver, gut and gastrocnemius by HPLC with a fluorimetric detection following derivatization with dansyl chloride [19].

**Determination of protein synthesis in tissues**

The protein synthesis rate was assessed by the flooding-dose method [14]. Briefly, frozen samples of gastrocnemius muscle, skin and kidneys were powdered in liquid nitrogen with a ball mill (Dangoumeau; Prolabo, Paris, France) and homogenized in 8 vols of 10 % trichloroacetic acid solution. Samples of gut, lung, liver and spleen were homogenized directly in ice-cold 10 % trichloroacetic acid solution with a Potter homogenizer. Tissue homogenates were centrifuged to separate the acid-soluble fraction. Supernatants containing free amino acids were purified by cation-exchange chromatography in mini-disposable columns (Amberlite AG50 × 8, 100–200 mesh, H⁺ form; Bio-Rad, Richmond, CA, U.S.A.). Amino acids were eluted with 4 M NH₄OH. After evaporation, the eluate was resuspended in 0.1 M HCl and used for measurement of free [\textsuperscript{13}C]valine enrichment by GLC-MS on a HP-5890 quadrupole spectrometer (Hewlett-Packard, Paris, France). Valine was measured as the tertiary isobutylidemethylsilyl derivative under electron-impact ionization. The remaining pellet containing proteins was washed twice with 10 % trichloroacetic acid solution, twice with a 0.2 M perchloric acid solution and solubilized in 0.3 M NaOH for 1 h at 37°C. An aliquot was then collected for protein assay [20] and the remainder was precipitated with 20 % 0.2 M perchloric acid overnight. After centrifugation, the supernatant was used to assay RNA [21], and the pellet was hydrolysed in 6 M HCl at 110°C for 48 h. HCl was evaporated, the pellet was diluted in 0.1 M HCl and desalted by cation-exchange chromatography. Amino acids were eluted with 4 M NH₄OH, dried by evaporation and resuspended in 0.1 M HCl. N-Acetylated derivatives of valine were prepared for measurement of [\textsuperscript{13}C]valine incorporated into proteins by GLC-combustion isotope ratio MS (Isochrom Fisons Instruments, Manchester, U.K.) as described by Yarasheski et al. [22].

Protein fractional synthesis rate (FSR) in tissues was calculated as described previously [9] from the following formula: \[
FSR = \left(\frac{E_n - E_o}{E_o} \right) \times 100/(E_i \times t),
\]
where \(E_n\) is the enrichment of protein-bound valine at the end of the incorporation period, \(E_o\) is the natural enrichment of protein-bound valine and \(E_i\) is the \textsuperscript{13}C enrichment of free valine calculated half-way between injection and killing. The protein absolute synthesis rate (ASR) was calculated as the product of the fractional synthesis rate and protein mass of the tissue.

**Data analysis**

Data are given as means ± S.E.M. with eight rats in each group. Differences between the two groups were tested using an unpaired bilateral homoschedastic Student’s \(t\) test. Differences for protein oxidation were tested using
Table 1  Blood parameters measured at 25 weeks

Values are means ± S.E.M. *P < 0.05 compared with control rats (n = 8 for both groups).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control rats</th>
<th>HLA-B27 rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma proteins (g/l)</td>
<td>55.2 ± 1.5</td>
<td>53.3 ± 1.5</td>
</tr>
<tr>
<td>Leucocytes (×10³/mm³)</td>
<td>10.5 ± 0.1</td>
<td>17.4 ± 0.6*</td>
</tr>
<tr>
<td>Red blood cells (×10³/mm³)</td>
<td>9.0 ± 0.2</td>
<td>7.8 ± 0.1*</td>
</tr>
<tr>
<td>Platelets (×10³/mm³)</td>
<td>357 ± 45</td>
<td>519 ± 17*</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>45.0 ± 1.3</td>
<td>39.8 ± 0.6*</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>16.5 ± 0.4</td>
<td>14.6 ± 0.2*</td>
</tr>
</tbody>
</table>

Dunnet’s test to take into account the influence of the day of the assay. P < 0.05 was considered significant.

RESULTS

Growth and food efficiency

Difference in the food intake among the groups varied over the time period of the experiment. Food intake was 9% lower in HLA-B27 rats than in control rats from 13- to 16-weeks old, similar in both groups from week 16 to 19, and again 11% lower in HLA-B27 rats in the 19- to 23-week period. During the whole period (13- to 23-weeks old), transgenic rats tended to have a lower growth rate than control animals (1.25 ± 0.08 g/day compared with 1.41 ± 0.03 g/day; P = 0.077). During the same period, food efficiency was not significantly different between the two groups (0.078 ± 0.005 g of growth/g of food intake compared with 0.081 ± 0.002 g of growth/g of food intake for HLA-B27 rats and control rats respectively). Between week 23 and 25, control rats were pair fed to HLA-B27 rats and ate 14.8 ± 0.4 g/day and 14.8 ± 0.1 g/day respectively. The final weights were 339 ± 8 g and 359 ± 8 g for HLA-B27 rats and control rats respectively.

Blood parameters

At 25-weeks old, HLA-B27 rats had an increased number of blood leucocytes and platelets, but fewer red cells, and decreased haemoglobin and haematocrit in comparison with control rats (Table 1). The plasma concentration of various acute-phase proteins fluctuated over time. Moreover, the pattern of variation observed was very different for each individual rat, because each animal underwent successive periods of increase and remission (Figure 1). At 25-weeks old, mean AGP and fibrinogen concentrations were higher in HLA-B27 rats (75.2 ± 10.1 mg/l compared with 21.7 ± 1.1 mg/l in controls for AGP, and 3.65 ± 0.30 g/l compared with 2.88 ± 0.12 g/l in controls for fibrinogen). The concentrations of A2M and albumin were not signif-

Figure 1  Time course of plasma concentration of acute-phase proteins

Individual data for four HLA-B27 rats are presented to illustrate the different profiles of variation observed.

Gastrointestinal inflammation

During the study, no clinical signs of arthritis or skin lesions were observed in HLA-B27 rats. In contrast, all HLA-B27 rats had loose stools. Inflammation was assessed initially by histology in the ileum and the colon. The ileum of HLA-B27 rats showed a normal structure, which did not differ from control animals (results not shown). In contrast, colonic tissues from HLA-B27 rats showed an enlargement of the mucosa due to an inflammatory infiltration of the lamina propria (Figure 2). There was a clear increased length of the crypts with a paucity of mucus-containing goblet cells. Inflammatory cells were granulocytes and mononuclear cells. Mild cellular inflammatory infiltration was also observed in the submucosa.

Gut inflammation was also estimated in the small intestine and colon by measuring the cytokine mRNA expression. The mRNA expression level of inflammatory cytokines (TNFα, IL-1α and IL-6) observed in the colon was higher in HLA-B27 rats than in control rats.
Protein synthesis during spontaneous inflammatory bowel disease in HLA-B27 rats

A

B

Figure 2  Histopathology of the colon in control (A) and HLA-B27 (B) rats
Slides were stained with haematoxylin and eosin. The original magnification was 100 ×. Overall thickness of the colonic mucosa was increased in HLA-B27 rats. An inflammatory infiltration of the lamina propria involved both mononuclear and polymorphonuclear cells. The length of crypts is increased and there is a diminution of mucus-containing goblet cells.

Figure 3  Semi-quantification of cytokine mRNA levels in the colon in HLA-B27 rats
Values are expressed as a percentage of the signals obtained in control rats. Error bars indicate S.E.M. * P < 0.01 compared with control rats (n = 8 for both groups).

Figure 4  Levels of GSH in the colon and the gastrocnemius muscle
Closed bars, HLA-B27 rats; open bars, control rats. GSH level was increased in the colon and reduced in the gastrocnemius muscle in HLA-B27 rats. Error bars indicate S.E.M. * P < 0.01 compared with control rats (n = 8 for both groups).

(Figure 3). At the same time, IL-10 mRNA expression level was weakly elevated in the colon HLA-B27 rats. No difference was found in the expression of these cytokines between the two groups either in the jejunum, ileum, liver or spleen.

Oxidative status
The level of oxidized proteins (carbonyl groups) increased slightly in the plasma of HLA-B27 rats (192.8 ± 2.8 d.p.m./µg of protein) compared with control rats (177.9 ± 3.4 d.p.m./µg of protein; P < 0.05). It was not significantly different between control rats and HLA-B27 rats in the extensor digitorum longus muscle (252.1 ± 1.7 d.p.m./µg of protein compared with 241.3 ± 1.7 d.p.m./µg of protein respectively). GSSG concentration was similar for both groups in blood, colon and gastrocnemius muscle, but GSSG was decreased by 20.4 % in the liver of HLA-B27 rats (0.19 ± 0.01 µmol/g compared with 0.15 ± 0.01 µmol/g). GSH concentration was similar in both groups in the blood, liver, ileum, jejunum and lungs, but was strongly increased in the colon of HLA-B27 rats (Figure 4). Conversely, the GSH concentration was decreased in the gastrocnemius muscle of HLA-B27 rats.

Protein synthesis in tissues
The mass of most tissues did not differ between HLA-B27 and control rats (Tables 2 and 3). However, the mass of all muscles was significantly lower in HLA-B27 rats than in control rats (1.68 ± 0.05 compared with 1.87 ± 0.05 for the gastrocnemius muscle, 484 ± 12 mg compared with 565 ± 14 mg for the tibialis muscle, 126 ± 2 mg...
Table 2 Protein synthesis in intestinal tissues of HLA-B27 and control rats
The units for $K_{RNA}$ are mg of protein synthesized/mg of RNA per day. Values are means ± S.E.M. *$P < 0.05$ compared with control rats (n = 8 for both groups).

<table>
<thead>
<tr>
<th>Intestinal tissue</th>
<th>Rats</th>
<th>Weight (g)</th>
<th>Proteins (mg)</th>
<th>FSR (%/day)</th>
<th>ASR (mg/day)</th>
<th>$K_{RNA}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>Control</td>
<td>0.76 ± 0.03</td>
<td>79.7 ± 3.7</td>
<td>99.6 ± 1.2</td>
<td>79.3 ± 3.8</td>
<td>15.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>HLA-B27</td>
<td>0.65 ± 0.03</td>
<td>69.1 ± 3.5</td>
<td>115.2 ± 3.9*</td>
<td>80.2 ± 5.9</td>
<td>16.8 ± 0.5*</td>
</tr>
<tr>
<td>Jejunum</td>
<td>Control</td>
<td>2.49 ± 0.11</td>
<td>251 ± 13</td>
<td>82.9 ± 4.9</td>
<td>209.9 ± 18.2</td>
<td>12.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>HLA-B27</td>
<td>2.71 ± 0.08</td>
<td>262 ± 12</td>
<td>99.1 ± 2.8*</td>
<td>259.4 ± 13.5*</td>
<td>14.3 ± 0.2*</td>
</tr>
<tr>
<td>Ileum</td>
<td>Control</td>
<td>2.23 ± 0.13</td>
<td>182 ± 12</td>
<td>66.2 ± 1.2</td>
<td>121.6 ± 9.9</td>
<td>11.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>HLA-B27</td>
<td>2.34 ± 0.11</td>
<td>198 ± 14</td>
<td>72.2 ± 1.7*</td>
<td>142.4 ± 8.9</td>
<td>12.4 ± 0.4*</td>
</tr>
<tr>
<td>Colon</td>
<td>Control</td>
<td>1.03 ± 0.02</td>
<td>82 ± 4</td>
<td>31.3 ± 1.3</td>
<td>25.6 ± 0.9</td>
<td>10.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>HLA-B27</td>
<td>1.14 ± 0.02</td>
<td>102 ± 4*</td>
<td>74.3 ± 9.6*</td>
<td>74.9 ± 7.8</td>
<td>13.9 ± 0.6*</td>
</tr>
</tbody>
</table>

Table 3 Protein synthesis in tissues of HLA-B27 and control rats
The units for $K_{RNA}$ are mg of protein synthesized/mg of RNA per day. Values are means ± S.E.M. *$P < 0.05$ compared with control rats (n = 8 for both groups). +, proteins in the skin are in grams.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rats</th>
<th>Weight (g)</th>
<th>Proteins (mg)</th>
<th>FSR (%/day)</th>
<th>ASR (mg/day)</th>
<th>$K_{RNA}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Control</td>
<td>9.44 ± 0.06</td>
<td>1736 ± 50</td>
<td>57.3 ± 1.5</td>
<td>1005 ± 49</td>
<td>12.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>HLA-B27</td>
<td>9.72 ± 0.11</td>
<td>1688 ± 68</td>
<td>67.5 ± 1.3*</td>
<td>1151 ± 46*</td>
<td>19.4 ± 0.5*</td>
</tr>
<tr>
<td>Gastrocnemius muscle</td>
<td>Control</td>
<td>1.87 ± 0.05</td>
<td>345 ± 11</td>
<td>5.93 ± 0.16</td>
<td>20.3 ± 0.4</td>
<td>11.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>HLA-B27</td>
<td>1.68 ± 0.05*</td>
<td>308 ± 8*</td>
<td>7.63 ± 23*</td>
<td>23.5 ± 1.0*</td>
<td>13.9 ± 0.3*</td>
</tr>
<tr>
<td>Skin</td>
<td>Control</td>
<td>6.50 ± 2.3</td>
<td>8.28 ± 0.44*</td>
<td>8.9 ± 0.4</td>
<td>737 ± 50</td>
<td>12.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>HLA-B27</td>
<td>6.48 ± 2.7</td>
<td>8.33 ± 0.41*</td>
<td>14.1 ± 0.7*</td>
<td>1158 ± 99*</td>
<td>13.1 ± 1.0</td>
</tr>
<tr>
<td>Spleen</td>
<td>Control</td>
<td>0.813 ± 0.023</td>
<td>105 ± 2</td>
<td>31.8 ± 1.0</td>
<td>33.3 ± 1.1</td>
<td>9.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>HLA-B27</td>
<td>0.710 ± 0.017</td>
<td>107 ± 3</td>
<td>41.0 ± 1.7*</td>
<td>44.0 ± 2.1*</td>
<td>9.9 ± 0.2*</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Control</td>
<td>2.00 ± 0.11</td>
<td>306 ± 9</td>
<td>28.9 ± 1.4</td>
<td>88.1 ± 4.7</td>
<td>10.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>HLA-B27</td>
<td>1.84 ± 0.06</td>
<td>283 ± 8</td>
<td>33.0 ± 0.5*</td>
<td>93.3 ± 3.8</td>
<td>11.6 ± 0.2*</td>
</tr>
<tr>
<td>Lungs</td>
<td>Control</td>
<td>1.39 ± 0.01</td>
<td>154 ± 7</td>
<td>26.9 ± 4.1</td>
<td>42.8 ± 8.3</td>
<td>9.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>HLA-B27</td>
<td>1.31 ± 0.01</td>
<td>143 ± 5</td>
<td>24.4 ± 0.3</td>
<td>34.8 ± 1.1</td>
<td>8.5 ± 0.3</td>
</tr>
</tbody>
</table>

compared with 147 ± 2 mg for the extensor digitorum longus muscle, and 98 ± 3 mg compared with 125 ± 3 mg for the soleus muscle. In all tissues studied, except the lungs, protein FSRs were higher in HLA-B27 rats than control rats (Tables 2 and 3). Protein synthesis was highly stimulated in the colon, where the FSR and ASR were increased 2.4- and 2.9-fold respectively. Protein ASR was also increased in HLA-B27 rats in the jejunum, liver, spleen, skin and gastrocnemius muscle by 23.6 %, 14.5 %, 28.4 %, 60.7 % and 22.3 % respectively. Histological evaluation of the muscle failed to show inflammatory infiltration (results not shown). The capacity for protein synthesis, indicated by the mg of RNA/g of protein ratio, was greater in the colon, gastrocnemius muscle, skin and spleen of HLA-B27 rats than in control rats (52.5 ± 4.1, 5.5 ± 0.1, 10.5 ± 0.5 and 41.8 ± 1.1 in HLA-B27 rats compared with 30.0 ± 0.9, 5.0 ± 0.1, 7.9 ± 0.9 and 35.2 ± 0.8 in controls respectively), but lower in liver (35.4 ± 1.0 in HLA-B27 rats compared with 45.7 ± 1.7 in controls). With the exception of the skin and the lungs, the ribosomal efficiency ($K_{RNA}$) increased in HLA-B27 rats (Tables 2 and 3), particularly in the colon and liver (by 32.4 % and 52.8 % respectively).

**DISCUSSION**

Chronic inflammation is associated with considerable disturbances of protein metabolism [9]. In the present study, tissue protein metabolism was explored for the first time in a model of spontaneous chronic inflammation that probably mimics human IBD. By comparing our data with the disruptions described previously in an induced model of IBD [9], we demonstrate that the modifications of protein synthesis are specific to the type of inflammation.

HLA-B27 rats of the 33-3 line spontaneously develop a chronic inflammatory disease that mainly affects the intestine [13]. The characteristics of the inflammation observed in the present study were similar to those described previously [11] for the same transgenic rats. In our experiments, all HLA-B27 rats had loose stools.

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Histological analysis of the intestinal tract characterized a prominent inflammation in the colon with a thickening of the mucosa linked to an increased depth of the crypts and extensive inflammatory infiltration of monocytes and granulocytes. There is also an apparent loss of mucous-secreting cells as reported previously [11]. All these data are in line with the previous characterization of colonic inflammation in these transgenic rats [11,12,23]. The inflammation of the colon in HLA-B27 rats was also characterized by an activation of the expression of inflammatory cytokines (TNF-α, IL-6 and IL-1β) and a low expression of the anti-inflammatory cytokine IL-10.

Such a Th1 profile is in general agreement with the profile found in other studies [13,23–25]. However, the relative increase of inflammatory cytokines varies from one study to another. We observed a predominant increase of IL-6 expression, whereas other authors [11,12,23] reported an elevated IL-6 expression in only some of the transgenic rats. These discrepancies in the cytokine pattern between the studies may reflect different disease activity or environmental factors. Actually, the prevalence of inflammation can range from 50 % [12] to 100 % [11,26]. This variability seems to be mainly related to environmental factors. Indeed, the germ-free state prevents the development of the inflammatory disease [27], and the severity of the lesions depends on the type of microflora in the gut of HLA-B27 rats [13,28–31]. Differences in bacterial flora could thus explain the variations observed between studies concerning the intensity of the inflammatory process and the cytokine response [32].

The systemic markers of inflammation were increased in our HLA-B27 rats. High leucocyte numbers have been described elsewhere [13]. The increased concentration of acute-phase proteins, AGP and fibrinogen presented for the first time, is in agreement with increased AGP mRNA levels reported previously [27]. However, the AGP levels were moderate when compared with the high values observed in acute diseases. This probably reflects a mild inflammatory state in the case of chronic diseases.

Inflammation can influence food intake and lead to growth disruption, and growth failure has been reported in adolescents with Crohn’s disease [33]. The growth rate of HLA-B27 rats has been documented poorly. In contrast with previous data indicating a normal growth in HLA-B27 rats [34], the growth rate in our HLA-B27 rats tended to be lower than that of control rats (−11 %). This growth retardation could be related to the small reduction of food intake, since food efficiency was similar in HLA-B27 rats and control rats.

HLA-B27 rats exhibited clear muscle atrophy, since all muscle masses were reduced in comparison with control animals. This model reproduces the muscle wasting generally observed in chronic intestinal diseases [2]. Such a muscle atrophy could be associated with disturbances in protein metabolism. We observed an activation of protein turnover in all organs studied except the lungs. This is in agreement with the increase in both protein synthesis and breakdown rates observed at the whole-body level in human IBD [4]. In patients, it was suggested that modifications of whole-body protein turnover could be linked to a multi-organ activation, rather than a consequence of local tissue changes at the inflammatory site [5]. Indeed, the increase in the fractional protein synthesis rate in the colon (2.6-fold) and liver (+30 %) was estimated to be insufficient to account for the increase observed at the whole-body level [5]. Since the increase in protein synthesis in the colon and liver of HLA-B27 rats was in the same range of magnitude (2.4-fold and 18 % respectively), the HLA-B27 rat seems a relevant tool to study the nature of protein disturbances linked to IBD.

Additional studies would be necessary to establish which parts of the colon (mucosa, submucosa and muscularis layer) are involved in the global increase of protein synthesis. However, the histological analysis strongly suggests the increased protein synthesis rate to be related to an elevated turnover of immune cells infiltrated in the lamina propria of HLA-B27 rats. Other cell types may be involved in the activation of protein turnover, but our data do not allow us to discriminate them.

Taking into account the contribution of muscle (approx. 45 %) to the total body mass in rats [35] and assuming that ASR observed in the gastrocnemius muscle was representative of other muscles, we evaluated the approximate muscle ASR at the whole-body level and the total protein synthesis in the ten organs studied. The contribution of each tissue to the total protein synthesis in the ten tissues studied was then calculated. Despite the high increase in protein ASR in the colon of HLA-B27, this activation (49 mg/day) was quantifiably weak compared with the total ASR increase in the ten tissues studied (990 mg/day). The liver, skin and muscle were mainly responsible for the ASR increase at the whole-body level. These three tissues accounted for 87 % of the increase observed in all the tissues studied. The increase of liver protein synthesis probably resulted from increased production of proteins associated with the inflammatory process such as acute-phase proteins. Despite the absence of obvious skin lesions in our study, the ASR increase in the skin of HLA-B27 rats may be a consequence of an epidermal inflammation, as described previously [36].

The activation of muscle protein synthesis (15.7 %) was unexpected, since this tissue exhibits a strong reduction in the synthesis rate in most acute pathological states such as sepsis and burn [37,38] as well as chronic colitis in DSS-treated rats [36]. The dietary restriction imposed on control rats, because of the pair feeding for the last 2 weeks of our study, may have blunted protein synthesis rate in these rats. The relative increase observed in muscle of transgenic rats may then be related to the pathology, independently of the food intake. The underlying mechanisms of such an activation of protein
turnover in the muscle are difficult to speculate in the present study. However, histological analysis of muscle failed to find any inflammatory feature in HLA-B27 rats. Therefore this effect is not linked to a local inflammation but could result from metabolic regulation in order to limit muscle loss. If the increase in protein synthesis observed in the gastrocnemius muscle of HLA-B27 rats is representative of protein metabolism perturbations in human disease, it suggests that muscle could also account for the protein turnover rise observed at the whole-body level in IBD patients. In any case, our data strongly suggest that peripheral organs play a role in the elevation of the whole-body protein synthesis during IBD.

Various models have been proposed to study IBDs. In most of them, inflammation is induced by the administration of chemical substances. In contrast, the pathology is spontaneous in HLA-B27 rats and our present results show important differences between these two types of models. Although both models reflect aspects of human IBD, they differ in the underlying mechanisms [39]. Thus the different results on protein metabolism observed during inflammation may differ because of the different molecular mechanisms implicated in intestinal inflammation. In addition, HLA-B27 rats show involvement of other inflammatory lesions, such as joints, skin or genital organs. In our experiments, we did not observe any of the important clinical signs in the joints or skin. However, alterations in physiology leading to inflammation are likely to have occurred. This may imply other repercussions on protein metabolism in the different tissues. At the systemic level, similar increases in the number of blood leucocytes were observed in both the HLA-B27 and DSS-treated rat models, but the profile of acute-phase proteins differed. During the chronic inflammation period, DSS-treated rats exhibited a minor change of AGP concentration, normal fibrinogen levels and, conversely, a marked increase in A2M concentration [9]. In contrast, HLA-B27 rats were mainly characterized by an increase in AGP and fibrinogen concentrations. It is not clear if such differences in the pattern of acute-phase proteins can influence protein metabolism, but the disturbances of protein metabolism were different in these two models. The magnitude of the increase of protein synthesis in DSS-treated rats was less pronounced in the colon [9,40] and more pronounced in the spleen [9] than in HLA-B27 rats. On this point, the HLA-B27 rat model seems closer to human IBD than DSS-treated rats. Another difference between these two models of IBD concerns the muscle. In DSS-treated rats, muscle atrophy occurred during the induction period by mechanisms probably similar to those described in acute injury, i.e. decreased protein synthesis and increased proteolysis [41]. During the chronic period, muscle mass was preserved due to a reduction of protein synthesis and proteolysis. Therefore muscles of DSS-treated rats were reported to be in a situation of low turnover, considered as a possible regulation aiming to limit muscle protein mass [9]. In contrast, in the spontaneous chronic disease developed by HLA-B27 rats, muscle atrophy occurred without any decrease in protein synthesis. Muscle protein loss is therefore due to increased proteolysis, which will be crucial to quantify in the future. In contrast with DSS-treated rats, the muscle was not preserved in HLA-B27 rats during the chronic inflammation. Thus the metabolic regulation of protein turnover seems to be different in these two models of IBD.

In HLA-B27 rats, the activation of muscle proteolysis could be related to oxidative stress. Muscle antioxidant defences were depressed, since GSH levels were low in HLA-B27 rats. The reduction of GSH in the muscle could be linked to its mobilization in the gut, as GSH is indispensable for the survival of macrophages in oxidative conditions [42]. Increased glutathione levels were also found in the colonic mucosa of rats with induced colitis, probably to counteract free radical production [43]. Decreased GSH levels in the muscle could reflect that the gut is preferentially protected during the injury and this should result in protein oxidation in the muscle. However, carboxyls were not increased in the muscle of HLA-B27 rats. The activation of muscle proteolysis could explain the elimination of the oxidized proteins before their accumulation. Such an activation of proteolysis in order to degrade oxidized proteins has already been described [44]. However, further investigations are needed to determine the precise extent of the increased proteolysis in muscles suggested by our results during IBD and to understand its implications.

In conclusion, the bowel disease present in HLA-B27 rats was associated with widespread modifications of protein metabolism in the colon, as for human IBD, but also in the skin, liver and muscle. The disturbances of protein synthesis in this model of spontaneous inflammation contrast with those reported in induced chronic inflammation. One major divergence consists of different mechanisms involved in the regulation of muscle atrophy. Different inflammatory pathologies can lead to different modifications of protein metabolism in tissues and, thus, probably require different nutritional supports. The existence of such differences between human IBDs needs to be clarified, but the present study highlights the possible need for various nutritional approaches in IBD to limit muscle wasting, depending on the underlying mechanisms of the pathology.

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