Does prolonged cycling of moderate intensity affect immune cell function?

J Scharhag, T Meyer, H H W Gabriel, B Schlick, O Faude, W Kindermann

Background: Prolonged exercise may induce temporary immunosuppression with a presumed increased susceptibility for infection. However, there are only few data on immune cell function after prolonged cycling at moderate intensities typical for road cycling training sessions.

Methods: The present study examined the influence on immune cell function of 4 h of cycling at a constant intensity of 70% of the individual anaerobic threshold. Interleukin-6 (IL-6) and C-reactive protein (CRP), leukocyte and lymphocyte populations, activities of natural killer (NK), neutrophils, and monocytes were examined before and after exercise, and also on a control day without exercise.

Results: Cycling for 4 h induced a moderate acute phase response with increases in IL-6 from 1.0 (SD 0.5) to 9.6 (5.6) pg/ml 1 h after exercise and CRP from 0.5 (SD 0.4) before to 1.8 (1.3) mg/l 1 day after exercise. Although absolute numbers of circulating NK cells, monocytes, and neutrophils increased during exercise, on a per cell basis NK cell activity, neutrophil and monocyte phagocytosis, and monocyte oxidative burst did not significantly change after exercise. However, a minor effect over time for neutrophil oxidative burst was noted, tending to decrease after exercise.

Conclusions: Prolonged cycling at moderate intensities does not seem to seriously alter the function of cells of the first line of defence. Therefore, the influence of a single typical road cycling training session on the immune system is only moderate and appears to be safe from an immunological point of view.

Training and competition sessions in road cycling usually last several hours, and it is well established that prolonged exercise may induce a temporary immunosuppression termed the “open window” with a presumed increased susceptibility for infection. However, relatively few data are available on immune cell function after prolonged cycling at moderate intensities typical for road cycling training sessions, as cyclists usually spend about 80% of their training time below 65% maximal oxygen uptake ($V_{O2max}$). Therefore, it was the aim of the present study to examine the influence of a standardised prolonged cycling session at moderate intensity on the function of immune cells which represent the so called “first line of defence”.

It was hypothesised that cycling for 4 h at a constant intensity of 70% of the individual anaerobic threshold (IAT) would initiate an acute phase response, characterised by an increase in interleukin-6 (IL-6) and C-reactive protein (CRP), and would weaken the activity of natural killer cells (NK cells), neutrophils, and monocytes (phagocytosis and oxidative burst).

Methods

Subjects

Twelve male competitive athletes (nine road cyclists, three triathletes; age 26 (SD 7) years, height 179 (5) cm, weight 71 (5) kg, body fat 11.6% (3.8%), heart volume 13.9 (1.6) ml/kg, who had been cycling for 6.5 (3.3) years and had spent 11 (3) h per week road training during the last season, were recruited for the study after giving their written informed consent. In addition to a physical examination, routine blood parameters were determined in each participant to exclude acute or chronic inflammatory diseases. An ECG at rest and during cycle ergometry as well as an echocardiography were performed to exclude cardiovascular abnormalities (ergometric data: maximal power output 365 (SD 35) W, maximal heart rate 191 (12) beats/min, maximal oxygen uptake 66 (6) ml/min/kg, power output at the IAT 261 (32) W and 3.7 (0.5) W/kg, respectively).

General design

After an incremental stage test to determine the IAT, a constant load trial and an examination on a control day without exercise were performed in randomised order.

Cycle ergometry

To determine the IAT by the method of Stegmann et al., an incremental multi-stage cycle ergometric test was started at a workload of 100 W and increased by 50 W every 3 min until exhaustion. Blood samples were taken from the hyperaemised earlobe at the end of each stage and 1, 3, 5, and 10 min after cessation of exercise to determine lactate concentrations (Super GL, Greiner Biochemica, Flacht, Germany). In addition, $V_{O2max}$ was measured by direct mixing chamber spirometry (Cortex MetaMax I, Leipzig, Germany).

Constant load trial and control day

The constant load trial of 4 h duration at an intensity of 70% IAT given in Watts was carried out on a 400 m track. Heart rate (Polar, Kempele, Finland) and oxygen consumption (Cortex MetaMax I) were recorded continuously. Subjects used their own bicycles which were equipped with an SRM powermeter (Schobarer, Jülich, Germany) to monitor workload. Blood samples from the hyperaemised earlobe to determine lactate concentrations were taken at rest and at the end of cycling after 4 h. Fluid intake was permitted every 30 min and registered exactly; only mineral water with no carbohydrates or energy content was supplied. The tests took

Abbreviations: AU, arbitrary unit; CRP, C-reactive protein; fMLP, formylated-1-methionyl-1-leucyl-1-phenylalanin; IAT, individual anaerobic threshold; IL-2, interleukin-2; IL-6, interleukin-6; NK cells, natural killer cells; NKCA, NK cell cytotoxic activity
of 50:1 and incubated in a final volume of 200 µl of effector and target cell suspension and Complete Medium. In the high control samples 30 µl interleukin-2 (IL-2) were added prior to the addition of the target cell suspension. In the negative control sample, no effector cells were added. All tubes were then centrifuged (3 min, 120 xg), incubated (120 min, humidified CO2 incubator), and placed on ice until flow cytometric analysis. To label permeabilised target cells, 50 µl DNA Staining Solution (Orpegen Pharma) were added to each sample and incubated (5 min, 0˚C) after vortexing. Dead and live target cells were determined by flow cytometry (FACSScan), which was performed within 30 min after addition of DNA Staining Solution. Per cent specific lysis was determined by subtracting the percentage of dead cells in the control sample tube from the percentage of killed target cells in the test samples (normal sample and high control sample with IL-2), and the number of NK cells needed to lyse one target cell were then calculated (needed NK cells = effector cells x% NK cells measured by flow cytometry/target cells x% specific lysis).

**Neutrophil and monocyte phagocytosis**

Neutrophil phagocytic activity was determined using a commercial test (PHAGOTEST; Orpegen Pharma), which allows the quantitative determination of fluorescein labelled opsonised *Escherichia coli* bacteria. Heparinised whole blood was vortexed and 100 µl was aliquoted on the bottom of a 5 ml tube and incubated in an ice bath for 10 min. Afterwards, 20 µl precooled *E. coli* bacteria were added and mixed. While the control samples remained on ice, the test samples were incubated for 10 min at 37˚C in a water bath. After incubation, samples were put on ice and 100 µl of iced cold Quenching Solution (Orpegen Pharma) and vortexed. Washing Solution (3 ml; Orpegen Pharma) was added and cells were spun down (5 min, 250 xg, 4˚C) twice and the supernatant discarded. After lysis was determined by subtracting the percentage of dead cells in the control sample tube from the percentage of killed target cells in the test samples (normal sample and high control sample with IL-2), and the number of NK cells needed to lyse one target cell were then calculated (needed NK cells = effector cells x% NK cells measured by flow cytometry/target cells x% specific lysis).

As described by Rothe,* 3 ml heparinised peripheral blood (10 IU/ml Na+ heparin) were layered onto Histopack 1077
Immune cell function after prolonged cycling

(Sigma) to allow sedimentation of erythrocytes at room temperature without centrifugation within 50 min. The leukocyte enriched supernatant was carefully harvested and put on ice. A volume of 1 ml Hank’s balanced salt solution (Sigma) containing $2 \times 10^6$ leukocytes was incubated at 37°C with 10 μl dihydrothodamine (100 μM/l), and 10 μl fMLP (10 μM/l) were added after 5 min. After 25 min of incubation samples were put on ice to stop the reaction and stained with propium iodide to differentiate living from dead cells. Flow cytometric measurements for gated neutrophils and monocytes, respectively, were made within 60 min after sample assessment.

CRP, IL-6, metabolic parameters, and hormones

CRP was determined turbidimetrically (Biomed, Oberschleißheim, Germany) and IL-6 by an enzyme linked immunosassay (R&D Systems, Minneapolis, MN, USA). Lactate, glucose (Super GL, Greiner Biochemica), glycerol, and triglycerides were determined enzymatically (Vitalab Lyte Analyzer, CIBA-Corning Diagnostics, Fernwald, Germany), and free fatty acids by photometry (Photometer 1101M, Eppendorf, Germany). Cortisol was determined by chemoluminescence (Magic Lyte Analyzer, CIBA-Corning Diagnostics, Fernwald, Germany), and epinephrine and norepinephrine radioenzymatically. 3

Statistics

All data are presented as means (SD). Differences between the constant load trial and the control day were tested using a two factor analysis of variance (mode and time), and the corresponding time points. All data are presented as means (SD). Differences between pre- and post-exercise values for metabolic parameters and hormones were tested using a paired Student’s $t$ test. Pearson’s coefficient of correlation was used to test correlations between selected variables. An α error <0.05 was considered as significant.

RESULTS

Metabolic parameters and hormones

All 12 cyclists finished the constant load trial of 4 h duration without any problems. The mean power output was 181 (SD 23) W, corresponding to a mean percentage of 72% (SD 5%) of the maximal heart rate and to a mean percentage of 59% (SD 6%) of the maximal oxygen consumption. Mean fluid consumption was 1.8 (SD 1) l mineral water. The results for metabolic parameters and hormones before and after exercise and the corresponding values of the control day are shown in table 1.

IL-6 and CRP

Significant increases in IL-6 from 1.0 (SD 0.5) before to 9.6 (5.6) pg/ml 1 h after exercise (fig 1A) and in CRP from 0.4 (0.5) before to 1.8 (1.3) mg/l 1 day after exercise (fig 1B) were noted. Significant correlations between IL-6 and the following parameters were found: CRP ($r = 0.71, p < 0.01$), epinephrine ($r = 0.70, p = 0.01$), norepinephrine ($r = 0.63, p = 0.03$), cortisol ($r = 0.71, p = 0.01$), and neutrophils ($r = 0.74, p = 0.006$). Furthermore, IL-6 correlated inversely to glucose ($r = -0.60, p = 0.04$). IL-6 did not correlate to

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mode</th>
<th>Before (SD)</th>
<th>End (SD)</th>
<th>1 h post (SD)</th>
<th>2 h post (SD)</th>
<th>1 day post (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes</td>
<td>70% IAT</td>
<td>4958 (1294)</td>
<td>11 186 (3497)</td>
<td>10 609 (3717)</td>
<td>10 746 (3576)</td>
<td>5479 (1460)</td>
</tr>
<tr>
<td>(cells/μl)</td>
<td>Control day</td>
<td>5133 (1061)</td>
<td>5331 (1193)</td>
<td>5650 (924)</td>
<td>6024 (1228)</td>
<td>5180 (1215)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>70% IAT</td>
<td>2949 (1122)</td>
<td>8716 (2863)</td>
<td>8847 (3483)</td>
<td>8389 (3035)</td>
<td>3340 (1096)</td>
</tr>
<tr>
<td>(cells/μl)</td>
<td>Control day</td>
<td>3186 (930)</td>
<td>3276 (894)</td>
<td>3450 (701)</td>
<td>3739 (898)</td>
<td>3140 (955)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>70% IAT</td>
<td>393 (155)</td>
<td>507 (161)</td>
<td>418 (184)</td>
<td>513 (185)</td>
<td>382 (177)</td>
</tr>
<tr>
<td>(cells/μl)</td>
<td>Control day</td>
<td>361 (144)</td>
<td>309 (115)</td>
<td>364 (73)</td>
<td>392 (110)</td>
<td>368 (72)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>70% IAT</td>
<td>1226 (467)</td>
<td>1 568 (604)</td>
<td>901 (312)</td>
<td>1 038 (272)</td>
<td>1 182 (387)</td>
</tr>
<tr>
<td>(cells/μl)</td>
<td>Control day</td>
<td>1 175 (256)</td>
<td>1 523 (382)</td>
<td>1 430 (321)</td>
<td>1 510 (397)</td>
<td>1 218 (382)</td>
</tr>
<tr>
<td>NK cells</td>
<td>70% IAT</td>
<td>167 (81)</td>
<td>294 (133)</td>
<td>57 (28)</td>
<td>103 (55)</td>
<td>157 (72)</td>
</tr>
<tr>
<td>(cells/μl)</td>
<td>Control day</td>
<td>170 (50)</td>
<td>220 (88)</td>
<td>171 (56)</td>
<td>203 (64)</td>
<td>159 (47)</td>
</tr>
</tbody>
</table>

See text for statistics.
concentrations of free fatty acids, triglycerol, or glycerol or to activities of NK cells, neutrophils, or monocytes.

**Leukocyte and lymphocyte subpopulations**

Exercise induced changes of leukocytes, neutrophils, monocytes, lymphocytes, and NK cells are shown in table 2. Significant increases were found for leukocytes and neutrophils after cessation of exercise (End), and 1 and 2 h after exercise (1 h post and 2 h post, respectively), which were ~2-fold for leukocytes and ~3-fold for neutrophils. The neutrophil count was significantly correlated to IL-6 \( (r = 0.74, p = 0.006) \) and, in trend, to cortisol \( (r = 0.54, p = 0.07) \). Furthermore, monocytes were significantly elevated after cessation of exercise as well as 2 h after exercise.

Lymphocytes were raised significantly on both the exercise day and the control day at the time exercise ended but were diminished significantly at 1 and 2 h after exercise (table 2). Counts of NK cells were raised significantly after cessation of exercise but were significantly decreased at 1 and 2 h after exercise (table 2). The rise in NK cells was strongly correlated to epinephrine \( (r = 0.74, p = 0.005) \), and moderately to norepinephrine \( (r = 0.65, p = 0.02) \) and IL-6 \( (r = 0.61, p = 0.04) \). Although CD3^+CD16^+CD94^+ and CD3^+CD16^+CD158a^+ lymphocytes were raised significantly at the end of exercise \( (p<0.05) \), they had dropped to significantly under pre-exercise values at 1 h and 2 h after exercise \( (p<0.05) \). CD94^+ lymphocytes/µL: 102 (SD 40) (before), 160 (77) (end), 30 (16) (1 h post), 50 (42) (2 h post), 94 (54) (1 day post); CD158a^+ lymphocytes/µL: 53 (19) (before), 100 (44) (after), 19 (8) (1 h post), 31 (23) (2 h post), 45 (10) (1 day post). The mean fluorescence intensities of CD3^−CD16^−CD94^+ and CD3^−CD16^−CD158a^+ lymphocytes remained unchanged.

**NK cytotoxic activity (NKCA)**

The number of NK cells needed to lyse one K562 target cell did not significantly change. This was also true for the interleukin-2 stimulated NKCA (fig 2).

**Neutrophil and monocyte oxidative burst**

The relative number of Rh123^+ neutrophils showed a significant different course between the exercise and the control days \( (p = 0.04) \) with a significant increase from 1 h post exercise to 1 day post exercise \( (p = 0.04); 33\% (SD 15\%) \) (before), 27\% (15\%) (1 h post), and 35\% (14\%) (1 day post) on the exercise day vs. 27\% (11\%) (before), 28\% (9\%) (1 h post), and 27\% (7\%) (1 day post) on the control day. The absolute number of Rh123^+ neutrophils was significantly increased after exercise (fig 3C).

The relative numbers of Rh123^+ monocytes did not significantly change: 23\% (SD 18\%) (before), 16\% (11\%) (1 h post), and 20\% (13\%) (1 day post) on the exercise day vs. 19\% (12\%) (before), 19\% (16\%) (1 h post), and 18\% (10\%) (1 day post) on the control day. The absolute number of Rh123^+ monocytes did not change (fig 3D). Although the mean fluorescence intensity of Rh123^+ monocytes did not show any significant changes, a significant effect of time for Rh123^+ neutrophils' mean fluorescence intensity was found \( (p = 0.038) \), tending to decrease after exercise: 36 (SD 17) arbitrary units (AU) (before), 27 (9) AU (1 h post), and 33 (11) AU (1 day post) on the exercise day vs. 34 (19) AU (before), 34 (10) AU (1 h post), and 43 (24) AU (1 day post) on the control day.

**DISCUSSION**

The present study aimed to examine immunological reactions and immune cell function after prolonged exercise in well trained cyclists under standardised but realistic training conditions. Intensity was determined by means of the IAT to guarantee uniform individual strain in cyclists. An intensity
of 70% IAT, which corresponded to 59% \( \text{VO}_{2\text{max}} \) in the present study, was chosen as cyclists usually spend about 80% of their training time below 65% \( \text{VO}_{2\text{max}} \). Therefore, the present findings are representative for immune reactions in well-trained and competitive cyclists during their training sessions.

**Acute phase response**

In the present study, a 3- to 4-fold increase in CRP and an almost 10-fold increase in IL-6 was observed, indicating a moderate acute phase response. This observation is in accordance with the results of previous studies which reported 3- to 10-fold increases in CRP after exercise.\(^{10,11}\) The increase in IL-6 is comparable to the increase after 2–2.5 h of cycle ergometric testing at 75% \( \text{VO}_{2\text{max}} \) reported recently,\(^{12,13}\) is less than after competitive cycling (250 km bicycle road race, duration 6.5 h),\(^{14}\) and is higher than after a single maximal or repetitive anaerobic cycle ergometric test.\(^{15}\) The higher increases in IL-6 reported for runners (up to 100-fold and more after a marathon race)\(^{16–20}\) can be explained by the higher mechanical muscular strain.

According to previous reports, the increase in IL-6 results from exercise induced decrease in blood glucose, as IL-6 is produced in the contracting muscle\(^{21,22}\) to regulate substrate delivery and especially to maintain the glucose supply to glycogen depleted muscles.\(^{23,24}\) Furthermore, the close relation between IL-6 and CRP in the present study supports exercise induced release of CRP through hepatocytes, which is induced by IL-6. But in contrast to Pedersen and Keller,\(^{23,25}\) we did not find a relation between the increase in IL-6 and an increase in free fatty acids, glycerol, or triglycerides caused by the lipolytic effects of IL-6. Furthermore, although IL-6 has additional anti-inflammatory properties\(^{21,22}\) and induces an increase in plasma cortisol,\(^{24,26,27}\) we did not find a relation to immune cell function.

**Exercise induced leukocytosis and immune cell function**

In a typical response to exercise, a more than 2-fold increase in the numbers of circulating leukocytes was observed, dominated by an almost 3-fold increase in neutrophils which resulted from IL-6 and cortisol mediated recruitment from the bone marrow as described previously.\(^{28–30}\) Although lymphocytes were not elevated significantly at the end of exercise, a significant increase in NK cells was noted. This increase is thought to be induced by the catecholamine mediated down regulation of adhesion molecules.\(^{31}\) The post exercise reductions observed in lymphocytes and NK cells, however, were negatively correlated with both epinephrine and IL-6 mediated increases in cortisol and were in accordance with previous reports.\(^{23,31}\)

But beyond alterations in absolute cell numbers, an attenuated immune function of different leukocyte subsets has been discussed in the literature,\(^{32–38}\) which might be responsible for greater susceptibility to opportunistic infections within the first hours after exercise and therefore are termed the “open window”\(^{2}\). As NK cells and macrophages represent the first line of defence, they have attracted particular interest.

**NK cell activity (NKCA)**

No differences in single cell NKCA were observed between before and after exercise, nor between the day of exercise and the control day. This was also true for the in vitro IL-2

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**Figure 3** Absolute numbers of phagocytising neutrophils and monocytes (A and B) and absolute numbers of Rh\(_{123}^+\) neutrophils and Rh\(_{123}^+\) monocytes (C and D) before (Before), 1 h and 1 day after (1 h post, 1 day post) 4 h of cycling at an intensity of 70% IAT as well as on the control day without exercise at the corresponding time points.
stimulated single cell NKCA, which, given that exercise increases the level of activity in part through IL-2 by an enhanced IL-2 receptor expression on NK cells, could have been more sensitive. Also the unchanged mean fluorescence intensities of CD3 CD16 CD19 1 and CD3 CD16 CD158 1 lymphocytes suggest that exercise had no influence on the expression levels on CD94 or CD158a receptors, which either activate or inhibit NK cell function.39 Taken together, single cell NKCA was unaffected.

The present observations accord well with previous reports on single cell NKCA using 51 Cr labelled K562 target cells.39 Nevertheless, they must be differentiated from an intensity dependent increase in total NKCA (reflecting the overall cytotoxic activity of a given population of peripheral blood mononuclear cells with an exercise induced higher proportion of NK cells) during or immediately after exercise.40 NK cell function expressed on a per cell basis has been reported to remain unchanged immediately after exercise in runners after 45 min to 2.5 h of treadmill running at intensities ranging from 50 to 80% VO2max or after repeated cycle ergometric testing.41 Therefore, these previous findings can be extended to even longer exercise bouts by the present results.

As single cell NKCA may not be affected even by prolonged exercise, it seems that augmented or attenuated immunocompetence of NK cells is more a matter of numerical redistribution than of single cell NKCA.

Neutrophil and monocyte phagocytosis and oxidative burst

Although exercise induced an increase in absolute numbers of phagocytising neutrophils (but not in monocytes), neither neutrophil nor monocyte phagocytosis on a per cell basis was affected by prolonged cycling in the present study. This finding is in accordance with the results of Malspica et al 18 who did not find an effect of exercise on the functional capacity of neutrophils due to small variations of epinephrine, whereas high concentrations of epinephrine, high intensity exercise, and exercise to exhaustion seem to alter neutrophil phagocytic capacity. Therefore, a typical cycling training session does not seem to alter the phagocytic capacity of monocytes or neutrophils.

Nevertheless, other mechanisms of microbial killing may be suppressed before the phagocytic capacity of macrophages is reduced. In the present study, neutrophil oxidative burst may have been more affected than other tested activities. Representing a semiquantitative estimate of the intracellular production of reactive oxygen intermediates per cell, the mean fluorescence intensity of Rh 123 neutrophils showed a significant effect over time and tended to be somewhat lower after exercise than before or the day after. This temporary affection presumably resulted from the IL-6 and cortisol induced increased influx of less mature neutrophils into the circulation from the bone marrow and the marginal pool as well as from direct cortisol effects on intracellular glucocorticoid binding sites. However, whether this small effect contributes to a higher susceptibility to infection in endurance athletes and therefore supports the open window theory remains unclear and needs further investigation. Taken together, although a minor temporary effect of neutrophil oxidative burst was found in the present study, the function of macrophages in the first line of defence. Therefore, the influence of a single typical road cycling training session on the immune system is only moderate and appears to be safe from an immunological point of view.

CONCLUSION

Prolonged cycling at moderate intensities induces a moderate acute phase response and possibly induces a moderate affection of neutrophil oxidative burst, whereas neutrophil and monocyte phagocytosis as well as NK cell activity remain unaffected. In conclusion, prolonged cycling at moderate intensities does not seem to seriously alter the function of cells in the first line of defence. Therefore, the influence of a single typical road cycling training session appears to be safe from an immunological point of view.

What is already known on this topic

Prolonged exercise may induce temporary immunosuppression with a presumed increased susceptibility for infection. However, little is known about immune cell function after prolonged cycling at moderate intensities.

What this study adds

Although prolonged exercise may induce temporary immunosuppression, a single typical road cycling training session appears to be safe from an immunological point of view.

REFERENCES

Immune cell function after prolonged cycling

Over the past 15 years, several authors have argued that a temporary depression in the immune response associated with a prolonged bout of intensive exercise may be sufficient to reduce the immediate defences of the body against viral infection, with a resultant increase in the incidence of upper respiratory infections. The present paper looks at the changes in various immune parameters seen when a small group of distance cyclists and triathletes cycle for 4 h at some 59% of their peak oxygen intake (70% of their individual anaerobic threshold). Findings are compared with data for the same individuals tested on a control day. The observed changes in immune function are small, and it is thus argued that distance training is safe from an immunological point of view. The crucial question is whether the exercise intensity chosen for the present experiments is representative of that adopted by athletes when they are undertaking serious training. The limited increases in epinephrine and norepinephrine concentrations show that subjects did not find the chosen protocol particularly stressful, and one might wonder whether many competitors would not train much closer to the intensity adopted during a road race (which can be as high as 95% rather than 70% of the ventilatory threshold for an event of 6–7 h duration). Nevertheless, amateur participants in marathon events may well train at only 70% of their anaerobic threshold, and assuming that one can apply the present findings to the response of low level performers, then the latter group seem unlikely to harm their immune systems during moderate training sessions.

REFERENCES


OVERVIEW OF THE EPIDEMIOLOGY OF EXERCISE IMMUNOLOGY

Chronic exercise and the immune system


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