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ИММУНОЛОГИЧЕСКИЙ КОНТЕКСТ ЛЕГКОЙ ТРАВМЫ ГОЛОВНОГО МОЗГА

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Резюме. Параметры нескольких популяций иммунных клеток (Т-клетки и макрофагальные субпопуляции) изучали в периферической крови и головном мозге на клинически релевантной модели легкой травмы головного мозга (ТГМ) у крыс. Популяция резидентных клеток врожденного иммунитета, состоящая из микроглии и астроцитов при локальном повреждении тканей вовлекается в реализацию воспалительного ответа. В ситуации с травмой показано также, что лейкоциты крови могут преодолевать гемато-энцефалический барьер и проникать в паренхиму головного мозга. Использовали методы проточной цитометрии и иммунофлуоресценции. Было отмечено повышение числа моноцитов и нейтрофилов в срок до 1 сут. после легкой ТГМ, с последующим снижением к концу сроков наблюдения. Было установлено, что число CD45$^+$ клеток и CD3$^+$Т-клеток снижалось через 1 день после ТГМ и постепенно росло до 14 сут., а процент CD4$^+$Т-клеток постоянно снижался с 7 до 14 сут., тогда как доля CD8$^+$Т-клеток повышалась с 7 до 14 сут. наблюдения. При легкой ТГМ у животных наблюдалось значительное снижение (в 3-10 раз) числа микрососудов с позитивной реакцией на SMI 71 на 8-й и 14-й день после травмы головы. Интенсивная окраска микрососудов на SMI 71 иногда сочеталась с расширением области позитивной реакции. Тонкие позитивные отложения продуктов наблюдадись в головном мозге здоровых животных вокруг стенок микрососудов. В поврежденной ткани головного мозга выявлялись макрофаги, позитивные по CD45^{high}/CD11b$^+$ популяции М1 на второй день после ТГМ и отмечались в значительном количестве на 8-14-й день. В мозолистом теле и ипсилатеральном участке стриатума содержание клеток, экспрессирующих CD16/11b$^+$, достигало максимума через 8 сут. после травмы, что коррелировало со снижением позитивного ответа на наличие эндотелиального антигена SMI 71. Таким образом, в остром периоде после легкой ТГМ в головном мозге определяется наличие иммунопатологических процессов, что может впоследствии вести к дизрегуляции нейроиммунных связей.

Ключевые слова: нейроиммунология, микроглия, врожденный и адаптивный иммунитет, легкая травма головного мозга

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IMMUNOLOGICAL CONTEXT OF MILD TRAUMATIC BRAIN INJURY

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Abstract. The parameters of several populations of immune cells (T cell populations, macrophage subpopulations) in peripheral blood and brain were studied in a clinically significant model of mild traumatic brain injury among rats. The population of resident cells of innate immunity of microglia and brain astrocytes with local tissue damage is involved in the implementation of the inflammatory response, it is also shown that in case of trauma, blood leukocytes can overcome the blood-brain barrier and penetrate the brain parenchyma. The methods of flow cytometry and immunofluorescence were used. An increase in the number of monocytes and neutrophils up to 1 day, after a mild traumatic brain injury (TBI) with a subsequent decrease to the end of the observation period was noticed. It was determined, that the number of $CD45^+$ cells, $CD3^+T$ cells decreased at 1 days post-injury (dpi), and rose slightly by 14 dpi, the percentage of $CD4^+T$ cells continuously declined from 7 to 14 dpi, while the percentage of $CD8^+T$ cells increased from 7 to 14 dpi. With mild traumatic brain injury in animals, a significant (3-10 times) decrease in the number of microvessels with a positive reaction to the presence of SMI 71 on the 8th and 14th day after head injury was observed. Intensive staining of SMI 71 microvessels was sometimes observed with an increase in the area of a positive reaction. Thin positive deposits of the reaction product are observed in the brain of healthy animals around the wall of the microvessel. In the damaged brain, $CD45^{high}/CD11b^+$ positive macrophages of the M1 subpopulation appeared in the brain tissue on the 2nd day after TBI and a significant amount was observed on the 8-14th day. In the corpus callosum and ipsilateral region of the striatum, the content of cells expressing $CD16/11b^+$ reached a maximum 8 days after TBI, which correlated with a decrease in the positive response to the presence of endothelial antigen SMI 71. Thus, in the acute period of mild TBI, the presence of neuroimmunopathological processes is determined in the brain, which can subsequently result to the dysregulation of neuroimmune connections.

Keywords: neuroimmunology, microglia, innate and adaptive immunity, mild traumatic brain injury

Introduction

A traumatic brain injury (TBI) is an injury that disrupts the normal function of the brain and can be caused by a bump, blow or jolt to the head, rapid acceleration air a penetrating head injury. The leading causes of TBI-related deaths are motor vehicle crashes, suicides and falls. The severity of TBIs is typically categorized using the Glasgow Coma Scale and can range from: (a) mild; (b) moderate; to (c) severe. The structural and functional damage in TBI lead to deficits resulting from both primary and secondary injury mechanisms [3]. The primary injury is the result of the immediate mechanical damage from direct contact and/or inertial forces to the brain that occurs at the moment of the traumatic impact. This damage can include direct neuronal, glial and other cellular damage, contusion, damage to blood vessels (hemorrhage) and axonal shearing [4]. Secondary injury evolves over minutes, to days, to months, to years after the primary injury and is the result of cascades of metabolic, cellular and molecular events. These occur concurrently with, and contribute to, alterations of endogenous neurochemical, inflammatory and neuroinflammatory mechanisms. Recent research has indicated that better diagnostic and assessment criteria are needed in the TBI field [12].

The central nervous system and cerebrospinal fluid is delimited by the blood-brain barrier (BBB), where a relatively autonomous immune barrier is functioning, being isolated from the general circulation. The morphofunctional basis of BBB are protein-tolerant neural tissue, commited progenitor stem cells, subpopulations of T and B lymphocytes, monocytes, macrophages and their products, microglia cells and natural killer cells. However the immune and nervous systems are intimately connected and each can directly influence the behavior of the other. In case of the nervous tissue damage, most immune reactions have functional, physiological, and sanogenic character, directed at removing the "heterologous" protein [5]. In particular, the sensitization of leukocytes to the brain antigen increases, the ratio of immunoregulatory cells changes, and circulating immune complexes from antibodies are formed. It has been shown that, with moderate and severe brain injuries, the formation of secondary immunodeficiency and a high level of neurosensibilization are based on dysfunction of regulatory subpopulations of cellular and humoral immunity [10].

Currently, it is consided that the early neuro-inflammatory response in TBI is primarily due to the activity of the components of the innate immune system [9, 16]. The population of resident cells of innate immunity of microglia and brain astrocytes with local tissue damage is involved in the implementation of the inflammatory response, it is also shown that in case of trauma, blood leukocytes can overcome BBB and penetrate the brain parenchyma [6]. Also, in case of the nerve tissue damage, the active synthesis and release of cytokines and chemokines, which activate cell receptors, is triggered, which, in the future, leads to local and systemic immune responses. The general effect of inflammatory mediators of innate immunity is aimed at limiting the spread of trauma and restoring homeostatic balance [7]. It has been shown that with neuroinflammation after a traumatic brain injury of mild degree, localization of immune cells in the area of peritrauma and beyond is noted [1]. The presence of peripheral inflammation can also affect the outcome of TBI and its immune components can penetrate the central nervous system, bypassing the BBB [7]. However, the implementation of functional activity and the role of innate immunity cells in the pathogenesis of traumatic brain injury (TBI) is not fully clear.

The aim of this study is to establish the degree of involvement of immune cells (macrophages, T cells, and microglia) in the acute period of an experimental mild traumatic brain injury.

Materials and methods

Young male Wistar rat (10 to 12-week-old) were obtained from Laboratory of "National Scientific Center of Marine Biology named after A.V. Zhirmunsky" (Vladivostok, RF). All animal experiments were approved by the Ethical Committee of Pacific State Medical University and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and the number of animals killed. Animals were divided into 3 groups: 1 — control, intact animals (10 pcs.); 2 — animals with modeling of a mild traumatic brain injury (25 pcs) and 3 — with modeling of a medium traumatic brain injury (25 pcs). All potentially painful interventions in the experiments, as well as euthanasia under combined injection anesthesia: zoletil 0.003 mg/g (Virbac, France), xylanite 0.008 mg/g (NITA-PHARM, Russia, Saratov), a solution of atropine sulfate 0.1% − 0.01 ml per 100 g.

A modified "weight-drop model" was used to reproduce a mild TBI. An installation that included a tripod, with a tube guiding the fall of the load, and a plastic container, on the bottom of which soft material was placed, was used to cause injury. To exclude resistance from a hard surface, the upper part of the container was covered with foil that can withstand the weight of the animal at rest, but tearing when struck on the head. A cargo weighing 200 g, with a cone height of 1 cm, a striker area of 3.14 mm², made of brass, which falls from a height of one meter onto the occipital region of the rat's head, was used. In this case, simultaneously with the blow, the animal freely falls into the container on the foam pillow from a height of 30 cm.

Mononuclear cells were stained with Rat T lymphocyte Cocktail, anti-rat CD3-APC, anti-rat CD4-PE, anti-rat CD8-FITC, and anti-rat CD11b-FITC, anti-rat CD45-PerCP, anti-rat CD86-PE, anti-rat CD206-APC (BD Biosciences, USA) following standard protocols and manufacturer's instructions. Data were obtained using a MACSQuant Analyzer 10 (MiltenyiBiotec, Germany), and analyzed Kaluza Analysis 2.1 software (Beckman Coulter, USA).

For histological examination of brain tissue, the samples were fixed in 4% neutral formalin, micropreparations were prepared according to the classical technology, followed by Nissl staining and hematoxylin-eosin. Immunohistochemistry. Brain sections were allowed to thaw and afterward incubated with blocking buffer (13 PBS containing 0.5% casein, Sigma-Aldrich) for 30 min. Primary antibody staining with monoclonal anti-Rat Blood-Brain Barrier Antibody, antiCD11b (Abcam, Cambridge, UK; 1:100), polyclonal rabbit antiCD45 (Invitrogen, USA; 1:200), polyclonal rabbit antiCD14 (Invitrogen, USA; 0.5-1 µg/mL), polyclonal rabbit antiCD16 (SP175, Abcam, USA; 1:150), polyclonal rabbit antiF4/80 (Invitrogen, USA; 1:500) diluted in PBS/0.5% casein was performed for 2 hrs followed by two times washing with the blocking solution. Then, sections were treated with the secondary antibody Fluore 488- and peroxidase conjugated anti-mouse IgG (Abcam, USA; 1:200) for 1.5 hrs. Slides were washed two times in PBS and two times in distilled water and finally covered with DAPI-containing mounting medium (Dianova, Hamburg, Germany). Images were acquired in 320 magnification by the fluorescence microscope Zeiss Axio Observer Z1 (Zeiss Micro-Imaging GmbH, Jena, Germany). All images were processed with NIS-Elements Imaging Software (Nikon, Japan) for automated cell counting. Mean cell counts were calculated from three random microscopic fields at X 200 magnification in the cortex, striatum, and corpus callosum (CC) of each section respectively, and three consecutive sections were analyzed for each brain. Data from these nine images per brain region are expressed as mean numbers of cells per square millimeter. Counts were made by an investigator who was masked to experimental group assignment (n = 4 to 5 animals per group).

Statistical analyses

All values are presented as mean±standard error of the mean. Data with two groups were analyzed with the Student's t-test (non-directional), and data with

repeated groups were analyzed with one-way analysis of variance and the Student Newman–Keuls test for post hoc comparisons. The Pearson product linear regression analysis was used to correlate the M1 or M2 phenotype with SMI-32 intensity. Differences were considered statistically significant at $p > 0.05$.

Results and discussion

Morphometric evaluation of the neocortex showed that the day after the implementation of mild TBI there was a narrowing of the capillaries, a significant increase in the content of hyperchromic neurons, including those with the presence of 2 nucleoli, was determined. After 7 days, there was a restoration of the lumen of the capillaries, an increase in the number of hypochromic neurons with an increase in the diameter of their nucleoli. By 14 days, an increase in the content of hyperchromic neurons with 2 nucleoli was detected again. In our opinion, hyperchromia of neurons on the first day of TBI is a sign of a decrease in the osmolarity of the cytoplasm as a result of potassium reduction, which is a consequence of narrowing of capillaries and ischemia. Moreover, in this period no obvious neurological disorders among animals were observed. By the end of the second week under TBI, the number of hyperchromic neurons sharply increases and the number of cells with 2 nucleoli remains high against the background of a high level of animal anxiety. A positive process of intracellular regeneration with activation of the processes in the nucleolar organizer was indicated by an increase in the number of intensively stained nucleoli in neurons in the recovery of ammonia silver on the 8th day after mild TBI. Thus, in experimental mild TBI signs a violation in the brain structure, indicating hypoxia.

The indicators of the number of segmented leukocytes – neutrophils and eosinophils in animals blood smears, stained with alizarin red C, two hours after TBI significantly increased. The indices for healthy animals were 39.1 ± 0.964 and 2.37 ± 0.258, and 2 hours after TBI – 59.2 ± 1.024 and $3.9\pm0.214\%$ ($p < 0.001$), respectively. After 24 hours, the number of segmented white blood cells decreased, and, against the background of leukopenia, remained a high content of white blood cells with active nucleoli. Determination of immune cell populations using flow cytometry revealed an insignificant increase in CD45+ cells compared with healthy rats. T lymphocyte subsets were characterized by the expression of cell surface markers: all T cells (CD3+), CD4+ or CD8+T cells (CD3+CD4+ or CD3+CD8+). Was determined, that the number of T cells decreased at 1 days post-injury (dpi), and rose slightly by 14 dpi. Quantitative data for the percentage of T cells are shown in Table 1. The percentage of CD4+T cells continuously declined from 7 to 14 dpi, while the percentage of CD8+T cells increased from 7 to 14 dpi (Table 1).

The BBB provides both passive and active protection to the brain, which can be significantly compromised following TBI [8]. In the case of TBI, post-injury BBB dysfunction can be biphasic [15] and occur as a direct result of the primary injury or as a delayed consequence of sustained inflammatory and cellular responses associated with the primary injury [8, 13]. The result of mild or moderate TBI may in BBB dysfunction characterized by a transient opening of endothelial tight junctions resulting in a temporary influx of inflammatory molecules and immune cells from the periphery [8, 13]. SMI 71 (anti-Rat Blood-Brain Barrier Antibody) is specific for a rat endothelial protein

TABLE 1. INDICATORS OF CELLS IN THE PERIPHERAL BLOOD AFTER MILD TRAUMATIC BRAIN INJURY

Indicators	Intact rats (n = 10)	Rats after Mild Traumatic Brain Injury				
		2 hours (n = 5)	1 days (n = 5)	2 days (n = 5)	7 days (n = 5)	14 days (n = 5)
Leukocyte, × 10⁹/l	6.5±0.21	12.70±0.21	6.50±0.21	6.50±0.21	6.50±0.21	6.50±0.21
Neutrophils: stab, % segmented nucleus, %	39.10±0.96 1.9±0.07 37.20±2.93	59.20±1.02 1.80±0.09 57.2±4.2	28.3±2.5 0.90±0.03 27.6±2.5	18.7±1.7 0.090±0.007 17.5±1.2	34.5±2.5 0.90±0.03 33.6±3.1	36.4±3.3 0 36.4±3.1
Eosinophils, %	2.370±0.258	3.90±0.21	1.8±0.7	1.68±0.50	2.80±0.21	2.3±0.5
Monocytes, %	2.80±0.33	3.2±0.4	2.8±0.3	1.9±0.3	2.10±0.04	2.6±0.5
CD45+, %	75.2±5.8	73.1±4.8	68.1±1.2	78.1±6.8	77.8±6.1	81.2±6.4
CD3+, %	44.3±1.9	41.1±3.7	39.0±1.2	58.0±4.2*	62.0±4.9*	68.0±7.2*
CD3+CD4+, %	29.5±2.1	28.7±2.7	27.9±2.4	25.4±1.8	18.3±1.7*	16.7±1.9*
CD3+CD8+, %	18.5±0.9	22.5±1.6	19.6±1.2	19.8±1.9	39.7±2.9*	41.3±1.9*
CD20+, %	12.6±1.8	11.8±1.5	14.7±1.2	16.2±1.4	26.5±1.7	32.5±1.6
CD16+, %	8.3±0.8	19.3±1.8*	21.2±1.8*	18.2±1.3*	10.1±1.1	12.1±1.4
CD14+, %	6.7±0.7	4.9±0.4	2.8±0.5	2.8±0.3	4.2±0.3	5.1±0.6

Note. *, values are reliable in comparison with indicators of intact animals, $p < 0.05$.

found in areas with blood-brain or blood-nerve barriers. It was shown, that its reactivity disappears in lesions of experimental allergic encephalomyelitis. With mild traumatic brain injury in animals, a significant (3-10 times) decrease in the number of microvessels with a positive reaction to the presence of SMI 71 on the 8th and 14th day after head injury was observed. Intensive staining of SMI 71 microvessels was sometimes observed with an increase in the area of a positive reaction. Thin positive deposits of the reaction product are observed in the brain of healthy animals around the wall of the microvessel. In general, these data indicate a slight violation of BBB permeability in the later stages after injury

Two distinct populations of macrophages signature genes are not only expressed in microglia/macrophages but also in other CNS cells or infiltrating [12]. A combination of CD11b and CD45 labeling can be used to distinguish microglia from macrophages: CD-45^{high}/CD11b$^+$ cells were considered macrophages and CD45^{low}/CD11b$^+$ cells were considered microglia. Cells of microglia have been classified into two subsets: pro-infammatory M1 and anti-infammatory M2. Expression of the marker CD16/11b macrophages M1 slightly increased in the incortex at day 1 after TBI and was significantly raised thereafter until day 3. In the corpus callosum areas and ipsilateral striatum, CD16/11b expression peaked bimodally at 3 days after TBI and was associated with microglia/macrophages. During this period, the reactivity of resident macrophages of neocotrex was mainly determined.

A comparative analysis of the number of cells expressing CD45 and F4/80 in the same areas of the brain cortex makes it possible to distinguish resident macrophages from cells of monocytic origin (peripheral). In the neocortex of control animals, macrophages with low expression of CD45 prevailed, whereas on the 8th day after TBI, an increased content of CD45^{high} cells was observed, which indicated an increase in the number of peripheral cells. It was also found that CD45^{high}/CD11b$^+$ positive macrophages of the M1 subpopulation appeared in the brain tissue on the 2nd day after TBI and a significant number of them was observed on the 8-14th day. In the corpus callosum and ipsilateral region of the striatum, the content of cells expressing CD16/11b reached a maximum 8 days after TBI, which correlated with a decrease in the positive response to the presence of endothelial antigen SMI71. It is known that, the destruction of neurons and astrocytes leads to a decrease

in the reactivity of this marker and, indeed, in the first 2 days after a mild TBI there was a slight decrease in the positive reaction to the presence of SIM71-positive microvessels and, on the contrary, on the 8th and 14th day of the experiment, the number of such microvessels significantly decreased (p = 0.01). These data indicates that delineating macrophages by an M1 (classically activated macrophages) or M2 phenotype in TBI obscures other macrophage subsets that may have distinct roles in the injury response.

So, the brain has a powerful innate immune system, consisting mainly of microglia, which is the first line of defense against infection or stroke. When these cells become activated in response to trauma, it signals an adaptive immune response, producing auto-antibodies to the central nervous system proteins and activating subgroups of T cells and attracting migration to the area of damage, where they can have a neurotoxic or neuroprotective effect. Macrophages can be activated when they enter the damaged brain and can acquire a cytotoxic phenotype. From such positions the manipulating the immune system has great promise as a therapeutic strategy. The development of new pharmacological agents holds great promise for ameliorating some of the most devastating neurologic and neurodegenerative diseases, including TBI. The robust immune response that is initiated following injury provides a host of potential targets that may dampen progressive neurodegeneration.

Conclusion

The results of our study prove the presence of neuroimmunopathological processes in the brain in mild experimental brain injury. The violation of the permeability of the blood-brain barrier, determined on the 8-14th day after the injury, is associated with an increase in the migration of immune cells in the brain, which can subsequently result in the dysregulation of neuroimmune bonds. In our opinion, the resulting primary alteration of neurons and the mediators, released at this moment, act as chemoattractants, contributing to an increase in the number of monocytic (alien) macrophages. Further studies need to be explored, including the mechanisms underlying changes of the M1/M2 balance, the specific roles of CD3$^+$/CD4$^+$/CD8$^+$T cells in the peripheral blood, and the possible relationship between immunity and the post-injury prognosis.

References

1. Bai R., Gao H., Han Z., Huang S., Ge X., Chen F., Lei P. Flow cytometric characterization of T cell subsets and microglia after repetitive mild traumatic brain injury in rats. *Neurochem. Res., 2017, Vol. 42, no 10, pp. 2892-2901.*

2. Brenner L.A., Vanderploeg R.D., Terrio H. Assessment and diagnosis of mild traumatic brain injury, posttraumatic stress disorder, and other polytrauma conditions: burden of adversity hypothesis. *Rehabil. Psychol., 2009, Vol. 54, pp. 239-246.*

3. Davis A.E. Mechanisms of traumatic brain injury: biomechanical, structural and cellular considerations. *Crit. Care Nurs. Q., 2000, Vol. 23, pp. 1-13.*

4. Cernak I. Animal models of head trauma. *NeuroRx, 2005, Vol. 2, pp. 410-422.*

5. Filiano A.J., Gadani S.P., Kipnis J. Interactions of innate and adaptive immunity in brain development and function. *Brain Res., 2015, Vol. 18, no. 1617, pp. 18-27.*

6. Girard S., Brough D., Lopez-Castejon G., Giles J., Rothwell N.J., Allan S.M. Microglia and macrophages differentially modulate cell death after brain injury caused by oxygen-glucose deprivation in organotypic brain slices. *Glia, 2013, Vol. 61, no. 5, pp. 813-824.*

7. Hellewell S.C., Morganti-Kossmann M.C. Guilty molecules, guilty minds? The conflicting roles of the innate immune response to traumatic brain injury. *Mediators Inflamm., 2012, 2012, 356494.* doi: 10.1155/2012/356494.

8. Jassam Y.N., Izzy S., Whalen M., McGavern D., El Khoury J. Neuroimmunology of traumatic brain injury: time for a paradigm shift. *Neuron, 2017, Vol. 95, no. 6, pp. 1246-1265.*

9. Kelso M.L., Gendelman H.E. Bridge between neuroimmunity and traumatic brain injury. *Curr. Pharm. Des., 2014, Vol. 20, no. 26, pp. 4284-98.*

10. Loane D.J., Kumar A. Microglia in the TBI brain: the good, the bad, and the dysregulated. *Exp. Neurol., 2016, Vol. 275, Pt 3, pp. 316-327.*

11. Masel B.E.; DeWitt D.S. Traumatic brain injury: a disease process, not an event. *J. Neurotrauma, 2010, Vol. 27, pp. 1529-1540.*

12. Mantovani A., Sica A., Sozzani S., Allavena P., Vecchi A., Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol., 2004, Vol. 25, no. 12, pp. 677-686.*

13. McKee C.A., Lukens J.R., Emerging roles for the immune system in traumatic brain injury. *Front. Immunol., 2016, Vol. 7, 556.* doi: 10.3389/fimmu.2016.00556.

14. Morganti-Kossmann M.C., Semple B.D., Hellewell S.C., Bye N., Ziebell J.M. The complexity of neuroinflammation consequent to traumatic brain injury: from research evidence to potential treatments. *Acta Neuropathol., 2019, Vol. 137, no. 5, pp. 731-755.*

15. Shlosberg D.B.M., Kaufer D., Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nat. Rev. Neurol., 2010, Vol. 6, no. 7, pp. 11-18.*

16. Wang G., Zhang J., Hu X., Zhang L., Mao L., Jiang X., Liou A.K., Leak R.K., Gao Y., Chen J. Microglia/macrophage polarization dynamics in white matter after traumatic brain injury. *J. Cereb. Blood Flow Metab., 2013, Vol. 33, no. 12, pp. 1864-1874.*

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