Systemic Diabetic Context-Induced Biochemical Periodontal Alterations in Children

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Our studies aimed the eventual correlations of chemokines and interleukins levels (IFN-γ, IL-10, IL-2, IL-4, IL-5 and TNF-α) in serum and gingival crevicular fluid of patients with periodontitis and juvenile insulin-dependent diabetes mellitus as compared to periodontal healthy subjects (32 vs. 24 children and adolescents, respectively). The diabetic body interferences with microbiological microenvironment imply serious efforts of the first one, resulting in a significant local and systemic secretion of IFN-γ. Thus, we can suggest that measurements of interferon levels in gingival crevicular fluid could be an important parameter in assessing the diabetes progress toward the destructive or inflammatory alterations of periodontal tissues. IL-10 associates an average level of secretion in blood, being decreased in the group with severe periodontal disease (the values in gingival crevicular fluid are inconsistent throughout the studied groups). This reduction in IL-10 secretion could play a role in driving the way for oral tissues toward the degradation in juvenile diabetes population. The levels of IL-2 are very small both in the periphery and in gingival crevicular fluid. These data suggest the possible existence of a local factor that blocks the secretion of this T-cell proliferation factor by lymphocytes and macrophages, especially in diabetic patients with periodontal alteration. We found relatively low IL-4 and IL-5 levels of secretion both in plasma and gingival crevicular fluid for all groups of patients, although a little bit larger in the case of IL-5. The pattern of distribution is quite irregular in all the experimental groups. The values of IL-4 in gingival crevicular fluid of patients with periodontitis were significantly higher than those of controls. The differences of cytokines secretions between groups of diabetic patients with or without periodontal alteration are more apparent in serum.

Keywords: juvenile insulin-dependent diabetes mellitus, periodontitis, chemokines, interleukins, serum, gingival crevicular fluid

Periodontitis is a chronic pathologic condition induced by oral cavity colonization with normal or abnormal microorganisms as bacteria, manifested as an immune reaction as inflammation. The local phenomena of oral microbiome toleration are very fine controlled by the secreted levels of interleukin 10 (IL-10), the same boarder process making the difference between normal resistance to microorganisms (e.g., bacteria) and immunopathologic response. The destructive processes (e.g., affecting alveolar bone) associated to periodontitis might be basically induced by the release of high amounts of T-helper cytokines. On contrary, it is not clear how T-helper activation intervenes and how IL-10 is intimately involved. The fine control of IL-10 is absolutely necessary for animal experimental-induced and human clinical establishing of periodontitis, modulating the levels of IL-17A as well as IL-17F. The highest protection in animal models and human established clinical periodontitis was associated to IL-17F concentrations, finely adjusted by IL-10 [1].

There exist large yet described variations in the expressed cytokine levels throughout the periodontitis disease. One such study evaluated the expressed levels of 19 cytokine genes, in close relationship with T-cell activation, covering all the phases of periodontitis, starting from initiation till the eventual resolution in rhesus monkeys. Furthermore, these cytokines' expressions were correlated to expressed destructive genes, involved in bone tissues alterations. The experimental model of a ligature-induced periodontitis was developed, and gingival samples were collected initially, 2 weeks, one month and 3 months after the induction of condition. The resolution phase was considered as being established 2 months after the ligatures removal. Quantitative real time polymerase chain reaction was used to quantify the expression of target genes starting from total RNA isolation. When periodontitis was initiated and progressing, the expression of IL-1β, IL-6, TGF-β and IL-21 was striking, whereas that of IL-18 and IL-25 was reduced. When we refer to resolution phase, IL-2 was augmented and IL-10 downregulated. The conclusion is that all the phases of periodontitis are including upregulated or downregulated expressions of genes associated to several types of T-helper cells [2].

The specific type of aggressive periodontitis could be considered a pathologic condition with multifactorial pathogeny, involving unclear relationships with lymphocytes from peripheral tissues. What type of immunodeficiency or altered immune functioning is characteristic? When a young group of 10 women with a clear diagnostic of aggressive periodontitis was evaluated, it was found through FACS a reduced population of switched memory B cells, that is (IgM(-), IgD(-), CD27(+)), in 90% of subjects. In contrast, activated B lymphocytes, as well as the naïve and transitional populations were almost normal. Furthermore, the serum concentrations of IgM, IgG, IgA antibodies as well as their subclasses were in physiological limits. When correlated, it seems to be clear that the reduced switched memory B lymphocytes are not able to alter the immunoglobulins serum concentrations or clinical development of aggressive periodontitis. The trigger of pro-inflammatory cascade could be represented by bacterial lipopolysaccharide (LPS), known to have the capacities to increase IL-1β and IL-8

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local levels and to reduce meanwhile the IL-4 concentrations. These alterations of cytokines releases are the magnets for lymphocytes migration toward the local pockets of aggressive periodontitis [3].

One of the most important risk factor incriminated in periodontitis, especially the aggressive form, is represented by TNF-α (-308) gene polymorphism. In various degrees, the TNF-α gene polymorphisms could be found in healthy persons as well as in chronic periodontitis. Is there a difference in the gingival crevicular fluid concentrations of TNF- α among the above mentioned persons? The very recent studies demonstrated that there are not significant differences in genotypes and alleles for TNF-α (-308) among patients with aggressive periodontitis, healthy persons as well as patients with chronic periodontitis. The TNF-α (-308) allele 2 was more frequent in persons with aggressive periodontitis as compared to chronic periodontitis or clinically healthy persons. Meanwhile, it is evident that the TNF-α (-308) allele 2 is more frequent correlated also with pockets depths >= 4 mm in the patients from this study [4].

Our studies aimed the eventual correlations of chemokines and interleukins levels of IFN-γ, IL-10, IL-2, IL-4, IL-5 and TNF-α in plasma and gingival crevicular fluid of patients with periodontitis and insulin-dependent diabetes mellitus.

Experimental part

Our study included 56 children and adolescents, 6-18 years old, divided in two large series: a) control group (24 children and adolescents) without general diseases; and b) insulin-dependent diabetes mellitus group (32 children and adolescents). The insulin-dependent diabetes mellitus group (b) was further subdivided taking into account the degree of metabolic control of diabetes in: 1)well and moderate controlled diabetes (HbA1 < 9%), 17 children and adolescents; and 2) weak controlled diabetes (HbA1 > 9%), 15 children and adolescents. The study included only the children and adolescents having the parents and grandparents from european (north caucasian) race, to reduce the genetic heterogeneity. Meanwhile, the study excluded from the beginning the children and adolescents with a recent history of hepatitis or antibiotics.

Evaluation of clinical indicators was achieved by oral examination in the dental office. Thus, plaque index (PI), gingival index (GI), probing depth (PD), bleeding on probing (BOP) and clinical attachment level (CAL) for all subjects included in the study were correlated with age, age of diabetes and its metabolic control (HbA1c values revealed).

Gingival crevicular fluid was harvested after bringing patients into the dental office, using the least aggressive method, and thus avoiding mechanical irritation. Strips of paper (Periopaper ProFlow Inc., Amityvile, NY) were inserted for 30 seconds in the gingival sulcus, interproximal, covering clinically relevant areas (redness and local changes of gum consistency). Harvesting was done after assessing the plaque index (PI) and strict control of plaque removal, isolation from saliva field and air drying of gingival sulcus. Strips contaminated with blood were discarded. Immediately after collection the samples were discharged in 250mm phosphate buffered saline (PBS), *p*H=7.4, at 4°C, and vortexed. Obtained solved gingival fluid was divided in 50 μL samples for flow cytometry.

The method used for determining the amounts of cytokines both in plasma and gingival crevicular fluid was flow cytometry. The levels of interleukins IL-2, IL-4, IL-5, IL-10, TNF-α and IFN-γ were measured by means of a kit multiplex: CBA (Cytometric Bead Array/Human Th1/Th2 cytokine array) for flow cytometry.

Meanwhile, plasma was separated from the same patients to determine the concentrations of pro- and anti-inflammatory cytokines, synthetized and released by leukocytes during the host inflammatory response.

The protocols involving human subjects were previously approved by the Ethics Committee of the *Grigore T. Popa* University of Medicine and Pharmacy from Iasi. When it was the case, the patients or their caregivers signed an informed consent, accepting the including into the research.

Results and discussions

The study results recorded correlations of periodontal clinical status with diabetes mellitus biological parameters as well as with host inflammatory response.

Thus, it can be seen that IFN-γ in the plasma of patients is found at a level that is moderately or significantly increased as compared to that of witnesses. It is also important to observe that the levels of IFN-γ recorded in gingival crevicular fluid are significantly lower in patients with insulin-dependent diabetes mellitus (fig. 1 and fig. 7). Such a behavior could be explained by alterations in oral microenvironment induced by the significantly higher values of gingival crevicular fluid glucose and urea in patients with diabetes. The changes in oral micro-environment create a favorable bacterial aggression with altered host immune response to periodontal pathogens.

IL-10 associates an average level of secretion in blood (fig. 2 and fig. 8), being decreased in the group with severe periodontal disease (the values in gingival crevicular fluid are inconsistent throughout the studied groups). This reduction in IL-10 secretion could play a role in driving the way for oral tissues toward the degradation in juvenile diabetes population.

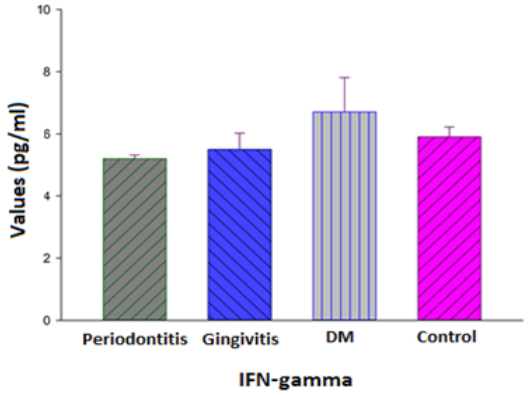

Fig. 1. IFN-γ in the plasma of patients with insulin-dependent diabetes mellitus (DM) is found at a level that is moderately or significantly increased as compared to that of control

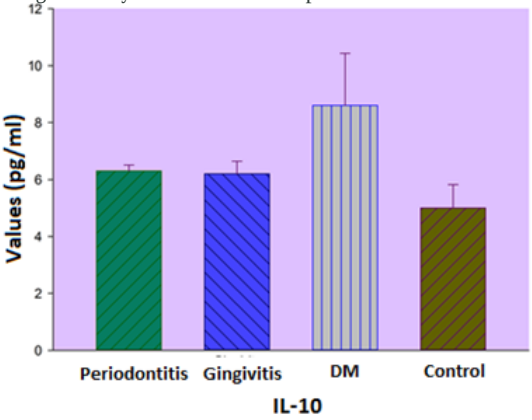

Fig. 2. IL-10 associates an average level of secretion in blood, being decreased in the group with severe periodontal disease

The levels of IL-2 are very small both in the periphery and in gingival crevicular fluid (fig. 3 and fig. 9). These data suggest the possible existence of a local factor that blocks the secretion of this T-cell proliferation factor by lymphocytes and macrophages, especially in diabetic patients with periodontal alteration.

We found relatively low IL-4 and IL-5 levels of secretion both in plasma and gingival crevicular fluid for all groups of patients, although a little bit larger in the case of IL-5 (fig. 4 and figure 10 as compared to fig. 5 and fig. 11). The pattern of distribution is quite irregular in all the experimental groups. The values of IL-4 in gingival crevicular fluid of patients with periodontitis were significantly higher than those of witnesses.

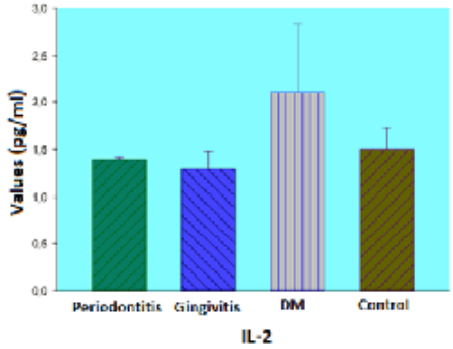

Fig. 3. The levels of IL-2 are very small in the periphery (plasma)

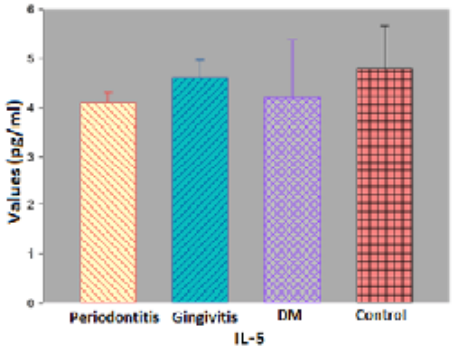

Fig. 4. The levels of IL-5 in periphery are reduced, although the highest as compared to IL-4 or IL-2

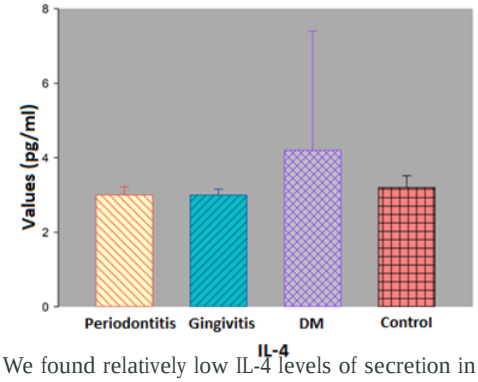

Fig. 5. We found relatively low IL-4 levels of secretion in plasma for all groups of patients

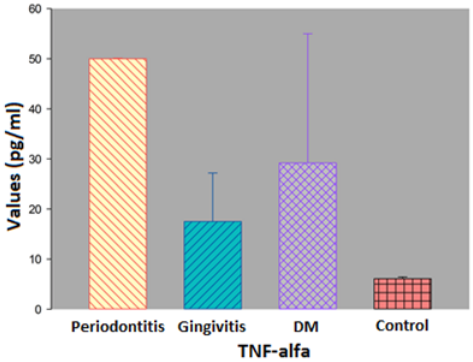

Fig. 6. The differences of TNF-α secretions between groups of diabetic patients with or without periodontal alterations are more apparent in the plasma.

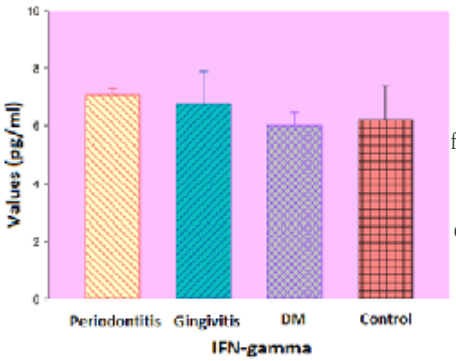

Fig. 7. The levels of IFN-γ recorded in gingival crevicular fluid are significantly lower in patients with insulin-dependent diabetes mellitus (DM)

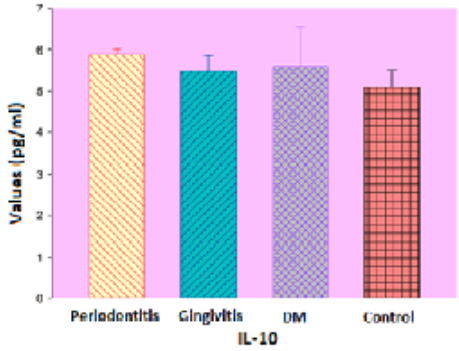

Fig. 8. The values of IL-10 in gingival crevicular fluid are inconsistent throughout the studied groups

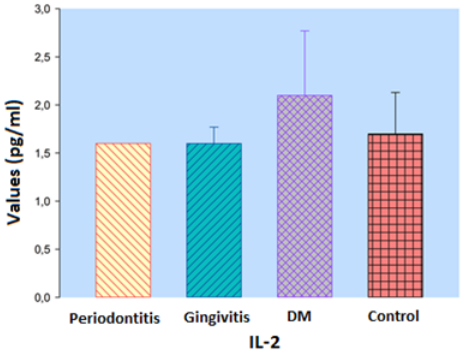

Fig. 9. The levels of IL-2 are very small also in gingival crevicular fluid

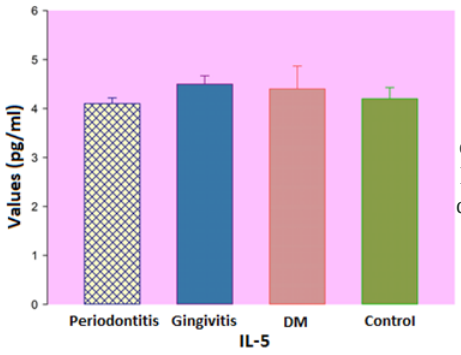

Fig. 10. IL-5 levels of secretion in gingival crevicular fluid for all groups of patients is really low. The pattern of distribution is quite irregular in all the experimental groups

The differences of cytokines secretions between groups of diabetic patients with or without periodontal alterations are more apparent in the plasma, considering TNF-α (fig. 6 and fig. 12), as well as IL-4, IL-5, and IL-2 (the latter one with absolute values of secretion lower than those of IL-4 and IL-5).

It is now considered that the so-called metabolic syndrome periodontitis are facets of the same condition, named inflammatory status. How different are the levels of cytokines in periphery and saliva in the northern part of India? TNF-α levels were higher in patients with metabolic syndrome and periodontitis, metabolic syndrome or periodontitis as compared to control groups. On contrary, plasmatic IL-10 was lower in patients with metabolic syndrome and periodontitis, metabolic syndrome or

periodontitis as compared to control groups. The saliva IL-10 was not significantly different for any of studied groups. The clear conclusion is that TNF-α could be correlated with clinical characteristics in persons associating metabolic syndrome and periodontitis [5,6].

There is large evidence that pre-diabetes is a risk factor for the development of periodontitis. But the mechanisms underlying such relationship are far of being revealed. Toll-like receptors, through NK-B activation, are involved in periodontitis pathogenesis and, thus, could be involved in pre-diabetic-enhanced inflammatory processes. The development of periodontal inflammation in rats with induced pre-diabetes is triggered through expression of toll-like receptors of type 2 and 4, and further activation of cascades involving NK-κB [7,8].

Hyperglycemia is inducing wide alterations of all metabolisms as well as of innate immune system functioning and reactivity. There are large evidences suggesting the deep involvement of local enhanced inflammatory responses in the alterations of periodontal tissues as a result of diabetes aggression. The released cytokines, matrix metalloproteinases, free radicals of oxygen are enhanced, having as result an aggressive local inflammatory response with the subsequent destruction of gingival tissues [9 -13]. Toll-like receptors (TLR) expression and functioning is also altered as a clear result of accumulating advanced glycation end-products, these ones altering all the immune responses, local or systemic ones. RANKL/osteoprotegerin axis is also a target for hyperglycemia during the non-catalytic attack of advanced glycation end-products on all tissues. Local migrated leukocytes induce high pressures on endothelial cells through an enhanced expression of integrins and selectins as well as an augmentation of IL-1, IL-6 and TNF-α secretion [14, 15].

When compared to healthy periodontal subjects, the patients with periodontitis present different amounts of released pro- or anti-inflammatory mediators throughout their gingival tissues and gingival crevicular fluid. Thus, IL-1α, IL-1β, IL-6, TNF-α and IFN-γ are increased in differently increased in different stages of periodontitis in adult patients. On the other side, the concentrations of IL-4 are really decreased in all periodontitis and gingivitis stages.

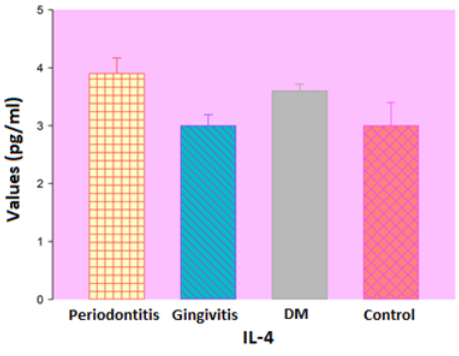

Fig. 11. The values of IL-4 in gingival crevicular fluid of patients with periodontitis were significantly higher than those of control

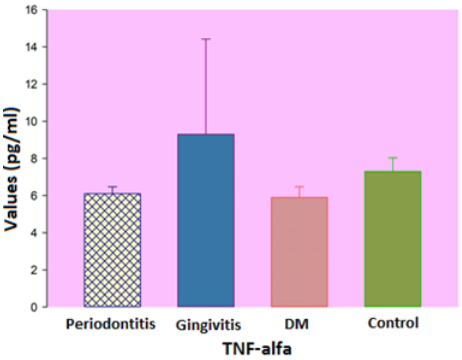

Fig. 12. The increase of TNF-α secretions is more evident in patients with gingivitis

All these above mentioned changes develop the basis for progressive destruction observed in periodontal diseases as a result of enhancement of inflammatory reactions, local or systemic ones [16].

Conclusions

The differences of cytokines secretions between groups of diabetic patients with or without periodontal alteration are more apparent in serum.

The diabetic body interferences with microbiological microenvironment imply serious efforts of the first one, resulting in a significant local and systemic secretion of chemokines and interleukins which are also enhancing alterations induced by the hyperactivity of immune system. In these cases, periodontitis could be the evolution result of hyperglycemia attack on gingival tissues.

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References

1. MORETTI, S., BARTOLOMMEI, L., GALOSI, C., RENGA, G., OIKONOMOU, V., ZAMPARINI, F., et al., J. Dent. Res., **94**, 9, 2015, p. 1267.
2. EBERSOLE, J.L., KIRAKODU, S., NOVAK, M.J., STROMBERG, A.J., SHEN, S., ORRACA, L., et al., J. Clin. Periodontol., **41**, 9, 2014, p. 853.
3. JENTSCH, H., PURSCHWITZ, R., STINGU, C.S., SCHMIDT, J., SACK, U., Laboratoriumsmedizin-J. Lab. Med., **39**, 3, 2015, p. 129.
4. YUCEL, O.O., BERKER, E., MESCI, L., FRATALAY, K., TEPE, E., TEZCAN, I., Cytokine, 72, 2, 2015, p.173.
5. CHAUHAN, A., YADAV, S.S., DWIDEDI, P., LAL, N., USMAN, K., KHATTRI, S., J. Clin. Lab. Anal., 30, 5, 2016, p. 649
6. LUCHIAN, I., MARTU, I., MARTU, C., GORIUC, A., BELDIMAN, A., MARTU, S., Rev. Chim. (Bucharest), **67**, 6, 2016, p. 1073.
7. HUANG, Y.L., GUO, W.H., ZENG, J., CHEN, G.Q., SUN, W.H., ZHANG, X.X., TIAN, W.D., J. Periodontol., 87, 5, 2016, p. E64.
8. BOARIU, M., MARINESCU, A., KRALEV, C., CLONDA, C., NEGRUTIU, M.L., Rev. Chim. (Bucharest), **65**, no. 12, 2014, p. 1477.
9. CORMOS, G., IONAS, M., BOTA, G., STEF, L., BOITOR, G.C., ORMENISAN, A., MARIS, M., MITARIU, M.C., Rev. Chim. (Bucharest), **67**, no. 9, 2016, p. 1697.
10. BOATCA, R.M., SCUTARIU, M.M., RUDNIC, I., MARTU-STEFANACHE, M.A., HURJUI, L., REZUS, E., MARTU, S., Rev. Chim. (Bucharest), **67**, no. 4, 2016, p. 741.
11. MARTU, I., LUCHIAN, I., GORIUC, A., TATARCIUC, M., IOANID, N., CIOLOCA, D.P., BOTNARIU, G.E., MARTU, C., Rev. Chim. (Bucharest), **67**, no. 6, 2016, p. 1378.
12. BARTOK, F.F., FORNA, N.C., Rev. Chim. (Bucharest), **67**, no. 9, 2016, p. 1722.
13. SOLOMON, S., URSARESCU, I., MARTU, A., LUCHIAN, I., AGOP-FORNA, D., MARTU, S., FORNA, N.C., Rev. Chim. (Bucharest), **66**, no. 8, 2015, p. 1166.
14. URSARESCU, I.G., MARTU-STEFANACHE, M.A., SOLOMON, S.M., PASARIN, L., BOATCA, R.M., CARUNTU, I.D., MARTU, S., Rev. Chim. (Bucharest), **67**, no. 2, 2016, p. 386.
15. SONNENSCHEIN, S.K., MEYLE, Y., Periodontology 2000, **69**,1, 2015, p. 221.
16. GURBAN, C.V., SUCIU, O., VLAIA, L., GOTIA, L.S., CORNIANU, M., ZAMBORI, C., MOTOC, M., VLAIA, V., VELEA, I., PRICOP, M., Rev. Chim. (Bucharest), **67**, no. 10, 2016, p. 1952.

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