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A Decrease in Cell-Mediated Immunity in Uremia Associated With an Increase in Activity of Suppressor Cells

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The graft-versus-host (GVH) reactivity of uremic and control spleen cells was studied by popliteal lymph node assay in the rat. The reaction evoked by cells from animals with severe uremia was conspicuously weaker than that evoked by control cells. The magnitude of the GVH reaction induced by control cells was directly proportional to dose, while with the uremic cells the same increases in dose led only to insignificant increases in the strength of the GVH reaction. When mixtures of syngeneic control and uremic cells were used, the GVH reactivity of the control cells was suppressed. The activity of uremic spleen cells can be enhanced (restored) by removal of the subpopulation of cells adherent to glass wool. The GVH reaction induced by uremic cells so treated became directly proportional to dose. The removal of the adherent cell population from the uremic spleen cell suspension also led to the disappearance of the suppressor effect in mixtures of control and uremic cells. These results indicate that a decrease in GVH activity of rat uremic spleen cells is due to an increase in suppressor cell activity in the uremic spleen cell population. (*Am J Pathol* 84:1-10, 1976)

CELL-MEDIATED IMMUNE REACTIONS in severe uremia have been reported as universally depressed.¹ Skin homograft survival was significantly prolonged in uremic patients² or in animals with experimentally produced chronic renal failure.³ Delayed hypersensitivity reactions were diminished in uremia⁴ as was the *in vivo* reactivity of uremic lymphocytes in the normal lymphocyte transfer reaction.^{5,6} Similarly, the *in vitro* reactivity of uremic lymphocytes in the mixed lymphocyte reaction⁷ and after phytohemagglutinin (PHA) stimulation^{8,9} was reduced. Recent

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studies in patients with well-compensated uremia have shown, however, that most cell-mediated reactions, except PHA responsiveness, appeared to be normal.¹⁰

Although uremia has been called "nature's immunosuppressive device,"¹¹ it should be noted that the depression of humoral responses in uremic states is much less pronounced than that of cell-mediated reactions. No effects have been reported on the responses to diphtheria¹² and tetanus¹³ toxoids and to endotoxin.¹⁴ The response of uremic rats to sheep red blood cells, an antigen strongly dependent on helper T-cell function, however, is reduced.¹⁵

The mechanism of this reduction of some immune reactions, and not others, by severe uremia is not well understood. The simplest explanation would be that there exists a selective reduction of a T-cell population. This idea is not supported by quantitative studies which, indeed, indicate that there is a disparity between the reduction of the response and the relatively unaffected number of T cells in uremia.¹⁶ With the discovery of a regulatory role of suppressor cells in immune reactions, the possibility exists that uremia leads to an increase in suppressor activity of certain cell subpopulations, resulting in depressed cell-mediated reactions. We decided to examine this possibility using controlled conditions such as those offered by the experimental model of the graft-versus-host reaction.

In this communication we demonstrate that splenic cells from rats with experimentally induced chronic renal insufficiency show a reduced ability to evoke the localized GVH reaction in nonuremic hosts and that this reduction is caused by an increase in suppressor activity of cells in the uremic spleen.

Materials and Methods

Inbred adult male Lewis (L) and (Lewis × Brown-Norway) F₁ hybrid rats (LBN) were obtained from Microbiological Associates, Walkersville, Md.

Chronic renal insufficiency was induced in Lewis male rats by partial (5/6) nephrectomy as described previously.^{3,15} Control animals were subjected to a sham operation (stripping of the kidney capsule). The development of uremia was monitored by the determination of blood urea nitrogen (BUN) levels.

The graft-versus-host reaction was measured by the popliteal lymph node assay method.¹⁷ Spleen cell suspensions from control and uremic Lewis rats were prepared under sterile conditions in saline. Cell viability was determined by a trypan blue dye exclusion test. The required number of viable cells was injected in a constant volume (0.5 ml) into the left footpad of the LBN recipient. The same volume of saline was injected into the right footpad. Seven days after injection, the popliteal lymph nodes were dissected and weighed to an accuracy of 0.1 mg. In each experiment, the control and uremic animals used as donors of spleen cells were of the same age.

The nonadherent spleen cell population was prepared according to the method of Folch and Waksman.¹⁸ A total number of 7×10^6 spleen cells suspended in 7 ml of Eagle's minimum essential medium supplemented with 10% of heat-inactivated fetal calf serum

was placed on a column containing 1 g of packed glass wool (Owens-Corning Fiberglass Corp., Corning, N. Y.). The column was incubated for 30 minutes at 37 C and then flushed with 30 ml of the same medium. The filtered suspension was then washed with saline and adjusted to the required cell density. The control cells were incubated for the same time in the same medium and washed with saline.

To determine the viability of spleen cells in tissue culture, aliquots of the spleen cell suspension were adjusted to a density of 10^6 cells/ml in Eagle's minimum essential medium supplemented with 20% of heat-inactivated fetal calf serum in capped tubes, and incubated at 37 C in an atmosphere of 5% CO_2 . At varying times the viability of cells was estimated by trypan blue dye exclusion.

Results

Comparison of GVH Reaction Induced by Control and Uremic Cells

The results shown in Table 1 indicate that when 4×10^7 viable spleen cells from control (sham-operated) Lewis rats were injected into LBN rats, the weight ratio of experimental and control lymph nodes was increased to 15.32. The same number of spleen cells from uremic rats caused a much lower increase (6.71). The difference in the strength of the GVH reaction induced by control and uremic cells was statistically highly significant ($P < 0.02$) in six different experiments. When BUN levels of the donors of uremic cells were lower than 50 mg%, the difference in the strength of the GVH reaction by control and uremic cells disappeared. The lower GVH reactivity of uremic spleen cells as compared to normal spleen cells is not due to the decreased viability of the uremic cell population. The percentages of nonviable cells detected in both uremic and nonuremic spleen cell populations are comparable and initially never exceed 10% of cells. When survival of the uremic and control cell populations was compared in tissue culture (Text-figure 1), no differences between the two cell populations were observed in the proportion of nonviable cells through 72 hours of incubation.

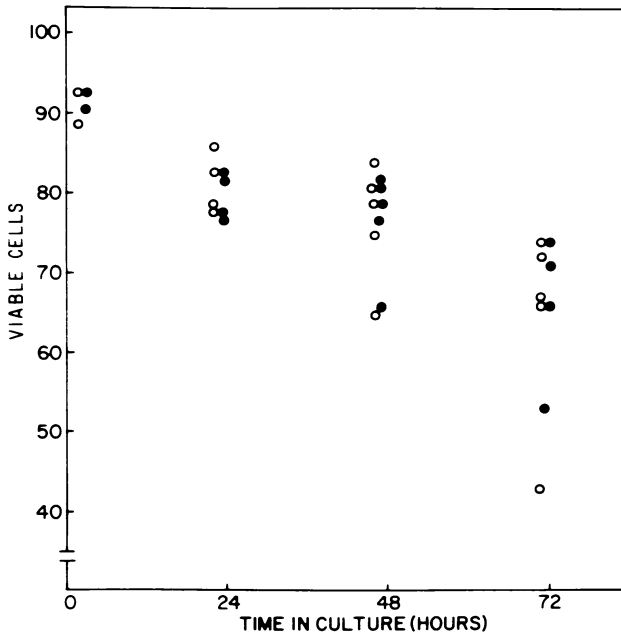
Reduced GVH Reactivity of Uremic Spleen Cells Is Due to a Suppressor Cell Effect

The results shown in Table 2 indicate that the magnitude of the local GVH reaction is dose dependent, but the relation of the increase in the

Table 1—Local Graft-Versus-Host Reaction by Control and Uremic Cells: Weight of Popliteal Lymph Nodes (mean \pm SE)

Control cells	Saline	Uremic cells	Saline
74.5 \pm 6.4	4.8 \pm 0.57	32.2 \pm 6.5	4.8 \pm 0.3

4×10^7 Lewis spleen cells were injected into the left footpad of LBN rats in 0.5 ml of saline; 0.5 ml of saline was injected into the right footpad. The results represent means of six separate experiments involving 3 animals each. The difference between the reactions induced by control and uremic cells is highly statistically significant ($P < 0.02$).



TEXT-FIGURE 1—Survival of control (*solid circles*) and uremic (*open circles*) spleen cells in tissue culture. The suspensions of spleen cells were incubated in Eagle's medium supplemented with 20% heat-inactivated fetal calf serum. Initial cell density was 10^6 cells/ml. At the times indicated, the viability of cells was determined by a trypan blue dye exclusion test.

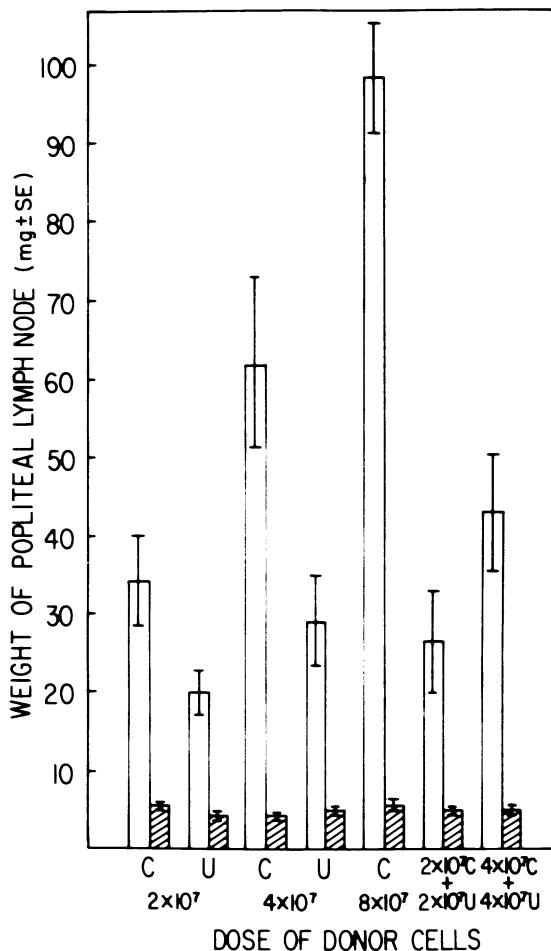
weight of the popliteal lymph node to the dose of injected cells depends on whether control or uremic cells are used. Injection of 2×10^7 control spleen cells resulted in an increase in weight of the regional lymph node to 33.6 ± 9.6 mg. When the dose of injected parental cells was doubled to 4×10^7 , the weight increased to 58.2 ± 8.3 . A second doubling of the inoculum dose resulted in a twofold further increase in weight of the regional node to 117 ± 9.7 mg. With the uremic cells the magnitude of reaction was different: the dose of 2×10^7 uremic cells caused the node weight to increase to 28.8 ± 5.6 mg. Doubling of the dose to 4×10^7 cells

Table 2—Dose Dependence of Local Graft-Versus-Host Reaction by Control and Uremic Spleen Cells

Cell dose	Weight of left popliteal lymph nodes (mean \pm SE)	
	Control cells	Uremic cells
2×10^7	33.6 ± 9.6 mg	28.8 ± 5.6 mg
4×10^7	58.2 ± 8.3 mg	32.0 ± 4.7 mg
8×10^7	117.0 ± 9.7 mg	36.0 ± 8.4 mg

The data represent means of results of two experiments each with a minimum of five recipients per group; uremic animals had BUN levels greater than 100 mg%. The weight of the right popliteal nodes did not exceed 6.1 mg.

TEXT-FIGURE 2—Graft-versus-host reaction produced by control, uremic, and a mixture of control and uremic cells. Data represent an average \pm SE from two independent experiments each involving a minimum of 5 control and uremic donors. The indicated numbers of cells were injected into the left footpad; the corresponding volume of saline was injected into the right footpad as a control. The BUN levels in uremic donors were greater than 80 mg%. C: control cells, U: uremic cells. Left node, open columns; right node, hatched columns.



resulted in an increase only to 32.0 ± 4.7 mg. Further doubling of the uremic cells led to an insignificant additional increase in popliteal lymph node weight to only 36.0 ± 8.4 mg. The dose-response relationship observed with uremic cells can be explained by the suppressor effect present in this cell population. To further explore this possibility, mixtures of parental control and uremic cells were injected into F_1 hybrid recipients. The results in Text-figure 2 indicate that the GVH reaction induced by injection of 2×10^7 control and 2×10^7 uremic cells is lower than that induced by 2×10^7 control spleen cells. A similar observation was made when a mixture of 4×10^7 control and 4×10^7 uremic cells was used. The difference between the GVH reaction induced by 4×10^7 control cells and by a mixture of 4×10^7 control and 4×10^7 uremic cells is statistically

significant ($P < 0.05$). As the control and uremic cells are syngeneic and therefore do not react one against another, the most likely explanation of the nonadditive activity of the mixtures of uremic and control spleen cells in the GVH reaction can be an increased suppressor effect in the uremic cell population. Accordingly, an experiment to remove suppressor cells was performed.

Increase of GVH Reactivity of Uremic Spleen Cells on Removal of an Adherent Cell Population

The suppressor cells from the rat spleen cell population can be removed by adherence to glass wool.¹⁸ If our assumption that the reduced GVH reactivity of uremic spleen cells is mediated by increased suppressor cell activity is correct, then the removal of that cell population should lead to enhancement of GVH reactivity of the uremic spleen cells. Filtration of both control and uremic spleen cells through a glass-wool column leads to removal of 30 to 40% of the cells. When such a filtered uremic spleen cell suspension is injected into footpads of F₁ hybrid recipients, a marked increase in the magnitude of the GVH reaction is observed (Table 3). The GVH reaction induced by filtered uremic spleen cells was stronger than that evoked by nonfiltered control spleen cells. There was a striking difference in the response in terms of the popliteal lymph node weight to doubling and redoubling of the dose of uremic spleen cells, depending on whether unfiltered or filtered cells were used. There was little increased response on doubling the dose in the former case, and a marked response on doubling the dose in the latter. The difference in magnitude of the reaction produced by filtered and nonfiltered uremic spleen cells with doses of 4×10^7 and 8×10^7 cells is statistically highly significant ($P < 0.01$). Removal of the adherent cells from the control spleen cell population also increases the intensity of the GVH reaction, but this increase is lower than that obtained with uremic cells.

Table 3—Effects of Removal of Glass-Adherent Subpopulation of Spleen Cells on Graft-Versus-Host Reaction

Cell dose	Weight of left popliteal lymph node (mean \pm SE)					
	Control cells			Uremic cells		
	Untreated (mg)	Filtered (mg)	Increase (%)	Untreated (mg)	Filtered (mg)	Increase (%)
2×10^7	33.9 \pm 2.1	42.0 \pm 6.3	23.9	25.9 \pm 3.1	33.0 \pm 4.8	27.4
4×10^7	74.2 \pm 5.8	84.2 \pm 12.1	13.5	31.2 \pm 9.3	83.4 \pm 9.6	167.3
8×10^7	136.8 \pm 14.4	172.1 \pm 8.3	25.8	34.3 \pm 7.1	155.8 \pm 18.3	354.2

The data represent means of two experiments each with a minimum of 5 recipients per group. Uremic donors had BUN levels greater than 90 mg%. The weights of the right popliteal nodes did not exceed 6.9 mg.

When a filtered uremic spleen cell population is injected admixed with a spleen cell population from control rats, the resulting GVH reaction is greatly increased; while injection of a mixture of 2×10^7 control cells and 2×10^7 uremic cells caused only a 6.1-fold increase in the weight of the popliteal lymph node, the injection of a mixture of the same number of nonadherent uremic cells and control cells increased the ratio 13.2 times (Table 4). A mixture of 4×10^7 control and 4×10^7 uremic cells caused only an eightfold increase in the regional lymph node weight while a mixture of the same number of control and filtered uremic cells caused a 17-fold weight increase. The differences in the strength of the GVH reaction induced by the mixtures of control and uremic and that of control and uremic filtered cells are highly statistically significant ($P < 0.02$). The experiments summarized in Tables 3 and 4 indicate that the GVH activity of uremic spleen cells can be markedly enhanced (restored) by removal of the subpopulation of cells that adhere to glass wool and that the suppressive effect of uremic cells on the GVH activity of control cells is removed by this procedure.

Discussion

This study demonstrates that the localized GVH reaction induced in the nonuremic host by uremic spleen cells is unusually weak. This reduction in magnitude of the response is not likely to be attributable to reduced viability of the uremic spleen cell population. The dose-response relationship of the GVH reaction evoked by uremic cells is best explained by a suppressor cell effect present in the uremic spleen cell population. This interpretation is further strengthened by results obtained with the mixtures of syngeneic control and uremic cells. The effects of the two kinds of cells in these mixtures are not only nonadditive, but the reaction by control cells is suppressed in mixtures with uremic cells. That suppressor cells can affect the GVH reaction was previously demonstrated,¹⁹ and in mice it was shown that spleen-localizing thymocytes were responsible.²⁰

Table 4—Disappearance of Suppressor Activity of Uremic Cells by Removal of Subpopulation of Adherent Cells

Cell inoculum	Weight of left popliteal lymph node (mean \pm SE)
2×10^7 control plus 2×10^7 uremic	34.2 ± 5.3 mg
2×10^7 control plus 2×10^7 filtered uremic	82.3 ± 14.6 mg
4×10^7 control plus 4×10^7 uremic	48.0 ± 7.1 mg
4×10^7 control plus 4×10^7 filtered uremic	109.3 ± 19.3 mg

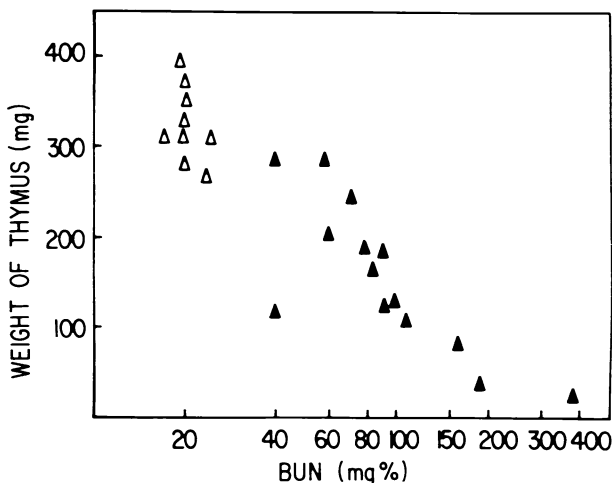
Uremic cells were obtained from donors with BUN levels greater than 90 mg%. A minimum of five recipients was in each group. The weight of the right popliteal nodes did not exceed 6.5 mg.

We demonstrate that the GVH reactivity of uremic spleen cells can be restored by removal of the subpopulation of cells adherent to glass wool. The number of cells removed by this procedure from both control and uremic cell populations is comparable, but the effects in each case are strikingly different. An explanation of the observed differences in reaction induced by nonfiltered and filtered uremic spleen cell populations by simple enrichment is unlikely. Moreover, enrichment cannot explain the results with the mixtures of control and uremic cells.

The observation of the absence of the suppressor effect following removal of the subpopulation of spleen cells adherent to glass wool is in good agreement with results previously established by other authors, indicating that the subpopulation of spleen cells adherent to glass wool is effective in the suppression of the mixed lymphocyte reaction.²¹

This population also seems to regulate the *in vitro* response to stimulation with phytohemagglutinin or concanavalin.¹⁸ It is therefore an attractive possibility to propose that the suppressor cell mechanism responsible for reduction of the GVH reactivity of uremic spleen cells might also be involved in the low response of uremic lymphocytes to lectins in our experimental model.²² Direct evidence for this point has to be provided, as it appears that the T-cell subpopulations responding, respectively, to stimulation by phytohemagglutinin and those responsible for the GVH reaction may be different.²³

Suppressor cell activity in the rat spleen increases following adult thymectomy.²⁴ It fits the results reported in this communication well because uremia in our model leads to a marked decrease in thymus weight



TEXT-FIGURE 3—Decrease of thymus weight with increasing severity of uremia. The animals were sacrificed by exsanguination; the thymuses were dissected, and weighed. (Control cells, open triangles; uremic cells, solid triangles)

(Text-figure 3); moreover, the thymus parenchyma in uremic rats is mostly replaced by connective tissue.

The results reported in this paper indicate that chronic renal insufficiency induced in rats leads directly or indirectly to disruption of the delicate balance of different cell subpopulations, to an increase in suppressor cell activity, and to a resulting decrease in graft-versus-host reactivity of uremic spleen cells.

References

1. Merrill JP: The immunologic capability of uremic patients. *Cancer Res* 28:1449-1451, 1968
2. Dammin GJ, Couch NP, Murray JE: Prolonged survival of skin homografts in uremic patients. *Ann NY Acad Sci* 64:967-976, 1956
3. Morrison AB, Maness K, Tawes R: Skin homograft survival in chronic renal insufficiency. *Arch Pathol* 75:139-143, 1963
4. Wilson WEC, Kirkpatrick CH, Talmage DW: Suppression of immunologic responsiveness in uremia. *Ann Intern Med* 62:1-14, 1965
5. Nelson SD, Bridges JM, McGeown MC: Comparison of lymphocyte-transfer test and white-blood-cell grouping for organ donor selection. *Lancet* 1:1359-1362, 1965
6. Carpenter CB, Glascock RJ, Gleason R, Corson JM, Merrill JP: The application of the normal lymphocyte transfer reaction to histocompatibility testing in man. *J Clin Invest* 45:1452-1466, 1966
7. Kasakura S, Lowenstein L: The effect of uremic blood on mixed leukocyte reactions and on cultures of leukocytes with phytohemagglutinin. *Transplantation* 5:283-289, 1967
8. Elves MW, Israels MCG, Collinge M: An assessment of the mixed leukocyte reaction in renal failure. *Lancet* 1:682-685, 1966
9. Huber H, Pastner D, Dittrich D, Braunsteiner H: In vitro reactivity of human lymphocytes in uraemia: A comparison with the impairment of delayed hypersensitivity. *Clin Exp Immunol* 5:75-82, 1969
10. Kauffman CA, Manzler AD, Phair JP: Cell-mediated immunity in patients on long term hemodialysis. *Clin Exp Immunol* 22:54-61, 1975
11. Lawrence HS: Uremia: Nature's immunosuppressive device. *Ann Intern Med* 62:166-170, 1965
12. Balch HH: The effect of severe battle injury and of post-traumatic renal failure on resistance to infection. *Ann Surg* 142:145-163, 1955
13. Stoloff IL, Stout R, Myerson RM, Havens WP Jr: Production of antibody in patients with uremia. *N Engl J Med* 259:320-323, 1958
14. Fink S, Mais RF: Normal cutaneous sensitivity to endotoxin in azotemia. *J Urol* 104:640-641, 1970
15. Raskova J, Morrison AB: Immunological responses to sheep red blood cells in experimentally induced chronic uremia of rats. *Immunol Commn* 2:607-614, 1973
16. Lopez C, Simmons RL, Touraine JL, Park BH, Kiszskiss DF, Najarian JS, Good RA: Discrepancy between PHA responsiveness and quantitative estimates of T-cell numbers in human peripheral blood during chronic renal failure and immunosuppression after transplantation. *Clin Immunol Immunopathol* 4:135-142, 1975
17. Ford WL, Burr W, Simonsen M: A lymph node weight assay for the graft-versus-host activity of rat lymphoid cells. *Transplantation* 10:258-266, 1970
18. Folch H, Waksman BH: The splenic suppressor cell. I. Activity of thymus-dependent adherent cells: Changes with age and stress. *J Immunol* 113:127-139, 1974

19. Gershon RK, Cohen P, Hencin R, Liebhaver SA: Suppressor T cells. *J Immunol* 108:586-590, 1972
20. Gershon RK, Lance EM, Kondo K: Immuno-regulatory role of spleen localizing thymocytes. *J Immunol* 112:546-554, 1974
21. Folch H, Waksman BH: The splenic suppressor cell. II. Suppression of the mixed lymphocyte reaction by thymus-dependent adherent cells. *J Immunol* 113:140-144, 1974
22. Raskova J, Morrison AB: Study of cell-mediated immune reactions in irradiated rats reconstituted with uremic bone marrow. *Fed Proc* 33:613, 1974 (Abstr)
23. Carr MC, Stites DP, Fudenberg HH: Dissociation of responses to phytohaemagglutinin and adult allogeneic lymphocytes in human foetal lymphoid tissues. *Nature [New Biol]* 241:279-281, 1973
24. Folch H, Waksman BH: In vitro responses of rat lymphocytes following adult thymectomy. I. Rapid decrease and recovery of responses to mitogens and hemiallogeneic cells. *J Immunol* 109:1046-1051, 1972

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