5-Aminosalicylic acid is a potent inhibitor of interleukin 1β production in organ culture of colonic biopsy specimens from patients with inflammatory bowel disease

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Abstract
Interleukin 1β in biopsy specimens from inflamed colonic mucosa of patients with active inflammatory bowel disease was studied. Compared with normal colonic mucosal biopsy specimens, a significantly greater amount of interleukin 1β was present in rectal mucosa before (median (range) 4·3 (2·0–11·8) v 119·2 (30·1–286·8) pg/mg; p<0·01) and produced during organ culture (39·1 (9·4–106·8) v 97·6 (28·2–991·6) pg/mg; p<0·01). Values of interleukin 1β after culture correlated with concentrations of thromboxane B_2. Organ culture of inflamed biopsy specimens in the presence of 5 aminosalicylic acid and dexamethasone reduced the amount of interleukin 1β detected. At the doses studied, 5 aminosalicylic acid also reduced the amount of leukotriene B_4 detected after culture.

Interleukin 1 has been shown to be a mediator of a number of inflammatory and immunological responses.[1-3] The precise mechanisms leading to inflammation in inflammatory bowel disease remain to be determined, however, and the mode of action of the therapeutically active drug 5-aminosalicylic acid is not clear. There are good reasons to believe that interleukin may be implicated in the pathogenesis of inflammatory bowel disease.[1] Its functional properties include T lymphocyte activation,[4] enhanced antibody synthesis by B lymphocytes,[5] and increased fibroblast proliferation.[6] It induces adhesion molecules on endothelial cells which facilitate migration of polymorphonuclear leucocytes and mononuclear cells to sites of inflammation.[7] It is also an endogenous pyrogen and induces synthesis of acute phase proteins by hepatocytes.[2]

Enhanced production of interleukin 1β by mononuclear cells isolated from colonic mucosa with active inflammatory bowel disease has been shown.[8] In the present study, we investigated whether the therapeutically active drug 5-aminosalicylic acid could inhibit interleukin 1β production by biopsy specimens from inflamed colons, and compared it with sulphapyridine (the therapeutically inactive moiety of sulphasalazine) and dexamethasone, whose effects on interleukin 1β synthesis by monocytes have previously been characterised.[9] We also evaluated the relation of interleukin 1β and thromboxane B_2 and studied the effect of 5-aminosalicylic acid on basal synthesis of eicosanoids.

Methods

PATIENTS
Twenty four patients with active ulcerative colitis (14 women, six men; median age 48·5 years (range 20–79)), two with active Crohn's disease (both women, aged 20 and 32 years), and 12 patients with normal colons (five women, seven men; median age 52·5 (range 27–81)) were studied. Biopsy specimens were obtained while these patients were undergoing colonoscopy for clinical reasons. The control group with normal colonic mucosa (confirmed on routine histology) were patients who were undergoing investigations for gastrointestinal symptoms and in whom no abnormality of the gastrointestinal tract was found.

Eight patients with inflammatory bowel disease were being treated with sulphasalazine, seven with mesalazine, six with oral prednisolone, four with steroid enemas, and four with azathioprine.

TISSUE AND CULTURE
Biopsy specimens (range of weight 1–9 mg) were usually obtained from the rectosigmoid region and for patients with inflamed colons, from areas of macroscopically homogeneously inflamed areas. The degree of inflammation was graded at endoscopy – grade 1 was mucosal oedema and loss of vascular pattern but no haemorrhage; grade 2, bleeding to light touch only; and grade 3, the presence of spontaneous bleeding seen ahead of the instrument at initial inspection.[10] For analysis, grades 2 and 3 were grouped together. For all the biopsy specimens from patients with inflammatory bowel disease, inflammation was confirmed on routine histology.

Some biopsy specimens were gently washed, dried, weighed, and homogenised without culture (in ultra turrax homogeniser, for one minute in 1 ml 10% fetal calf serum/RPMI). After centrifugation, the supernatant was stored at −70°C.

Other biopsy specimens were cultured as previously described.[11] They were placed on stainless steel mesh over a culture dish (Falcon) containing culture medium. The latter was made up of 10% fetal calf serum (FCS/RPMI; Gibco), supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 μg/ml gentamicin.

The biopsy specimens were cultured in the presence or absence of the drugs under investiga-

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tion. The culture dishes were placed in a sealed chamber equilibrated with 95% O_2/5% CO_2 and incubated at 37°C. The culture medium was changed after one hour and the culture continued for a further 23 hours. After this period, excess moisture was removed on blotting paper and the biopsy specimen was weighed and homogenised as described above. The culture medium was centifuged and supernatant stored.

Tissue homogenates and media in which biopsy specimens were cultured were assayed for interleukin 1β. The total amount of the cytokine after culture (homogenate+medium) was determined and expressed as pg/mg tissue.

All the samples for assay of interleukin 1β were in 10% FCS/RPMI and were stored in aliquots in cryotubes (Nunc) at −70°C. Our previous studies have shown that interleukin 1β is stable for long periods when stored in this manner.

Values of thromboxane B_2 and leukotriene B_4 in media of organ cultures were also determined.

DRUGS
5-Aminosalicylic acid and sulphapyridine were dissolved in dimethyl sulphoxide and dexamethasone in culture medium. Control cultures contained dimethyl sulphoxide or culture medium only.

ASSAY OF INTERLEUKIN 1β, THROMBOXANE B_2, AND LEUKOTRIENE B_4
Interleukin 1β was assayed using ELISA (Cistron Biotechnology/Laboratory Impex). The inter and intra-assay variation in our laboratory is less than 14%. None of the drugs studied interfered with the assay.

Thromboxane B_2 and leukotriene B_4 were assayed by radioimmunoassay using specific antibodies.[12] Antibodies against thromboxane B_2 and leukotriene B_4 were gifts from Dr L Levine (Waltham, Mass, USA), and Dr A W Ford-Hutchinson (Merk Frost, Canada), respectively.

Assays of thromboxane B_2 were performed after 100 fold dilution of samples with Tris buffer at pH 7·4 (thus after dilution the concentration of FCS was 0·1%). Standards were always prepared in Tris buffer containing similar amounts of FCS and RPMI to that present in the final dilution of samples. In any case, there was no difference in the standard curves obtained in the presence or absence of the very small amounts of FCS and RPMI.

Drugs used in this study did not interfere with the assay of thromboxane B_2 or leukotriene B_4.

Samples from experiments studying the effect of drugs, were always assayed together in batches.

STATISTICS
One way analysis of variance and Student's t test were used for normally distributed data (expressed as mean (SEM)). The Mann Whitney U test and Wilcoxon test for paired data were used for data that were not normally distributed (expressed as median (range)). For correlation, the Rank Spearman test was used.

Results
The variation in the amount of interleukin 1β present in pairs of biopsy specimens from the same areas of the colon was less than 20%. Initial studies showed that the highest values of total interleukin 1β were detected after culture for 24 hours.

Haematoxylin and eosin stained sections showed that viability of the biopsy specimens (as shown by lack of epithelial cell desquamation) was preserved after 24 hours' culture but not after 48 hours' culture. Culture (for 24 hours) in the presence of drugs studied did not affect the viability.

The viability (as assessed by exclusion of trypan blue) of peripheral blood mononuclear cells was not affected by the drugs studied (mean (SD) viability in medium only: 92·6 (7·8)% v viability in the presence of drugs: 90·7 (6·5)%).

STUDIES ON INTERLEUKIN 1β
There was significantly more interleukin 1β in homogenates of biopsy specimens, from colons with active inflammatory bowel disease before culture (median (range): 4·3 (2·0–11·8) v 119·2 (30·1–286·8) pg/mg; p<0·01 (Mann Whitney U test); Fig 1). All patients in the inflammatory bowel disease group had grade 2/3 inflammation. One patient was on no treatment (value of interleukin 1β, 134·3 pg/mg), three were being treated with sulphasalazine or mesalazine only (median (range) value of interleukin 1β: 71·0 (30·1–104·1) pg/mg), and four were being treated with oral prednisolone, with or without a 5-aminosalicylic compound or azathioprine (median (range) value of interleukin 1β: 152·8 (53·1–286·8) pg/mg).

In paired biopsy specimens from same areas of the colon (six normal, nine inflamed), more interleukin 1β was detected after culture (total of homogenate and culture) than before, suggesting that synthesis of the cytokine occurred in culture
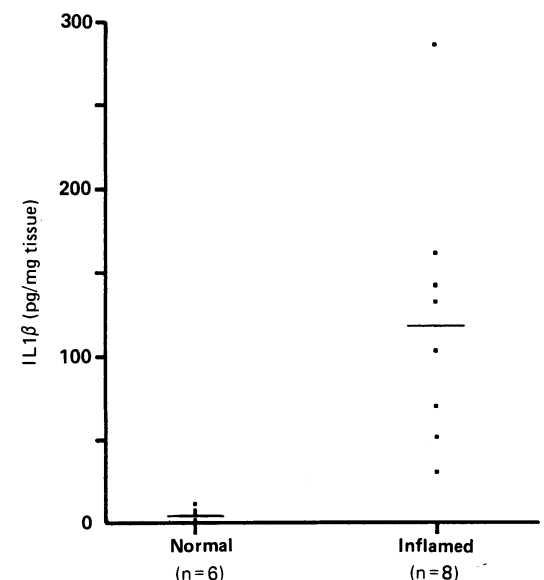

Figure 1: Interleukin 1β (IL1β) in homogenates of biopsy specimens (before culture) from normal colons and colons with active inflammatory bowel disease (7 ulcerative colitis, 1 Crohn's disease). Median represented by horizontal bars. Normal v inflamed: p<0·01 (Mann-Whitney U test).
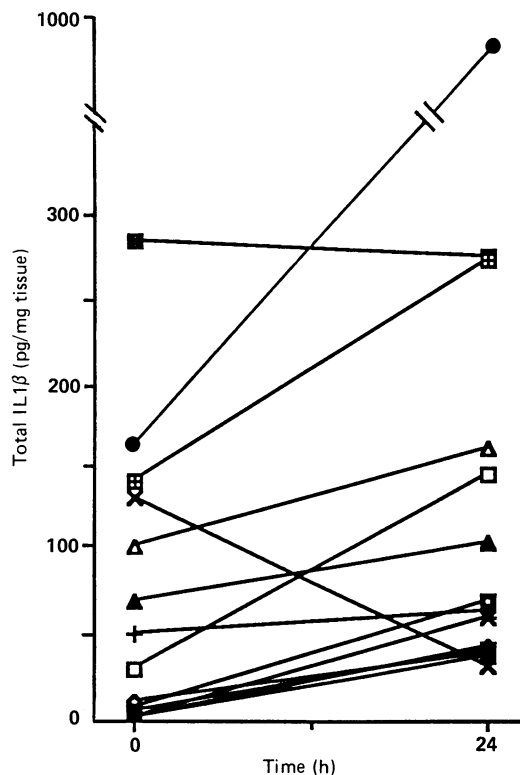
Figure 4: Total interleukin 1β (IL1β) (represented as mean (SEM)% of paired control biopsy specimens) detected after organ culture of inflamed tissue with different concentrations of 5-aminosalicylic acid (5ASA) (p<0·01; analysis of variance).

Pairs of biopsy specimens studied at each concentration: 10 μg/ml–6; 25 μg/ml –8; 50 μg/ml –8; 100 μg/ml–9. Mean (SEM) total interleukin 1β (IL1β) present in control biopsy specimens was 166·8 (72·6) pg/mg tissue.

Figure 2: Interleukin 1β (IL1β) present before (0 hours) and after (24 hours) culture of paired biopsy specimens from same areas of colon (0 hours v 24 hours, p=0·006; Wilcoxon test for paired data).

(median increase after culture: 388·7% (range: −75·5–2975); Fig 2).

Significantly greater amounts of total inter-

leukin 1β were present after organ culture of biopsy specimens from inflamed colons than from normal colons (median (range): 97·6 (28·2–991·6) v 39·1 (9·4–106·8); p<0·01 (Mann Whitney U test); Fig 3). Six of the inflamed colons had grade 1 inflammation and 14 had grade 2/3 inflammation. Values of interleukin 1β in mucosa were higher in the latter group than in the former (median (range): grade 1 – 62·0 (28·6–125·8) pg/mg v grade 2/3 – 128·8 (28·2–991·6) pg/mg; p<0·05 (Mann Whitney U test)).

Two patients with inflammatory bowel disease were receiving no treatment (values 32·9 and 34·1 pg/mg), nine (three grade 1, six grade 2/3) were taking sulphasalazine or mesalazine only, and seven (one grade 1, six grade 2/3) were being treated with oral prednisolone with or without a 5-aminosalicylic acid compound or azathioprine. There was no significant difference in the amount of interleukin 1β present between the group taking a 5-aminosalicylic acid compound only and the one being treated with oral prednisolone (median(range): 106·3 (28·2–228·5) pg/ml v 125·8 (84·2–991·6) pg/ml respectively).

EFFECT OF DRUGS

The drug studies were performed in organ cultures of inflamed biopsy specimens only. Drugs in varying concentrations were added to organ cultures and compared with paired biopsy specimens (obtained from the same colon) cultured in the same amount of dimethyl sulphoxide (10 μl) only or medium only. Total interleukin 1β (homogenate plus medium) present after culture in the presence of drugs was expressed as a percentage of the total intereukin 1β in the control biopsy specimen – that is, paired biopsy specimen cultured in the absence of the drug.

Studies with aminosalicylic acid

In organ cultures in the presence of 5-amino-salicylic acid there was significant reduction in the amount of total interleukin 1β detected (Fig 4). This reduction occurred in a dose dependent
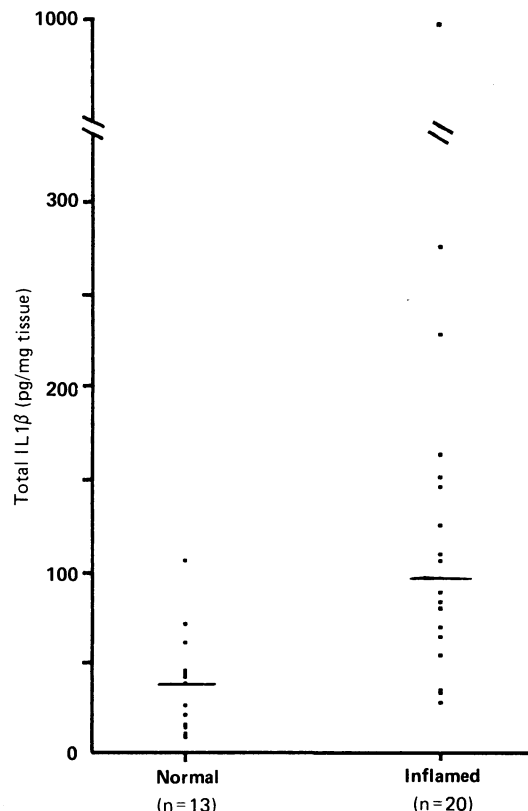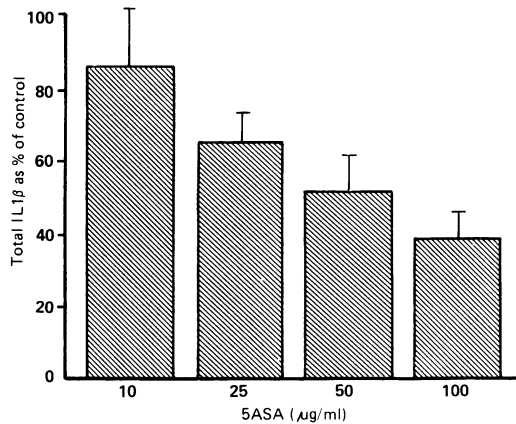

Figure 3: Total interleukin 1β (IL1β) detected after culture of biopsy specimens from normal and inflamed colons (19 ulcerative colitis, 1 Crohn's disease). Median represented by horizontal bars. Normal v inflamed: p<0·01 (Mann Whitney U test).
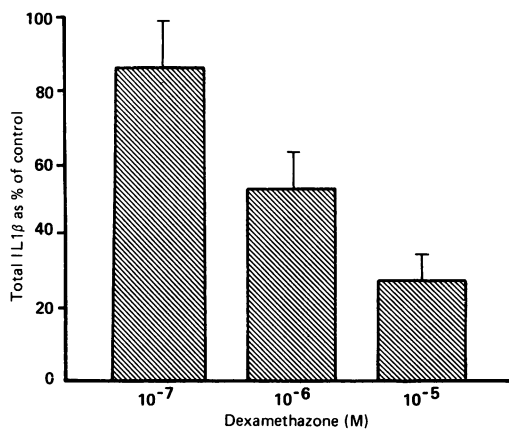
Figure 5: Total interleukin 1β (IL1β) (represented as mean (SEM)% of paired control biopsy specimens) detected after culture of inflamed biopsy specimens with different concentrations of dexamethasone (p<0·01; analysis of variance).

Pairs of biopsy specimens studied at each concentration: $10^{-7} M-4; 10^{-6} M-5; 10^{-5} M-5.$

Mean (SEM) total IL1β present in control biopsy specimens was 139·1 (80·5) pg/mg tissue.

fashion. The minimum concentration of 5-aminosalicylic acid at which significant reduction occurred was 25 μg/ml, when (mean (SEM)) 65 (7·5)% of the control biopsy specimen interleukin 1β was detected (p=0·002, Student's paired t test). Maximal reduction occurred at a concentration of 100 μg/ml (0·65 mM), when (mean (SEM)) 38·0 (16·2)% of the control biopsy specimen total 1β interleukin was detected (p<0·001; Student's paired t test).

Studies with sulphapyridine

There was no significant difference in the amount of interleukin 1β detected when biopsy specimens were cultured in the presence of sulphapyridine at concentrations of 50 μg/ml (0·2 mM; mean 133·5 (19·9)% of control biopsy specimen interleukin 1β detected) and 100 μg/ml (0·4 mM; mean 105·3 (26·1)% of control biopsy specimen interleukin 1 detected).

Studies with dexamethasone

Culture of biopsy specimens in the presence of dexamethasone caused a dose related reduction in the total amount of interleukin 1β detected (Fig 5). A significant reduction occurred at a concentration of 10^{-6} M when a mean 52·4 (11·7)% of the control biopsy specimen interleukin 1β was detected (p=0·015; Student's paired t test). In the presence of 10^{-5} M dexamethasone, a mean 27·8 (7·1)% of control biopsy specimen interleukin 1β was detected (p=0·002; Student's paired t test).

THROMBOXANE B_2 SYNTHESIS

5-Aminosalicylic acid, at concentrations of 50 and 100 μg/ml, did not make any significant difference to the amount of thromboxane B_2 present in media (mean (SEM)): 89·1 (27·4)% and 71·7 (18·1)% of control respectively) of organ cultures of inflamed biopsy specimens.

Significantly more thromboxane B_2 was detected in the medium of organ cultures of inflamed biopsy specimens compared with

medium of normal specimens (median (range): 365·6 (138·3–915) pg/mg v 114 (39·8–302·4) pg/mg; p<0·05).

There was a significant correlation between values of thromboxane B_2 and interleukin 1β in medium (of organ cultures) (r_s=0·76; p=0·011 (n=13)). There was also a significant correlation between values of thromboxane B_2 in medium and the total amount of interleukin 1β (in homogenate+medium) after organ culture (r_s=0·71; p=0·015 (n=13)).

LEUKOTRIENE B_4 SYNTHESIS

In some studies, the effect of 5-aminosalicylic acid on leukotriene B_4 synthesis was investigated. There was significant reduction in the amount of leukotriene B_4 detected in media of inflamed biopsy specimens in organ culture in the presence of 50 and 100 μg/ml of 5-aminosalicylic acid (mean (SEM): 50 μg/ml – 27·4 (21·6)% of control; p=0·043; 100 μg/ml – 21·3 (7·2)% of control; p<0·001). In the absence of any drug, the mean amount of leukotriene B_4 present in the media of the inflamed biopsy specimens was 34·8 (15·1) pg/mg tissue.

Discussion

The wide variety in the biological effects of interleukin 1 suggests that it may have a role in the mediation of inflammation and tissue damage in inflammatory bowel disease. Enhanced production of this cytokine by mononuclear cells from colonic mucosa in active inflammatory bowel disease has been shown.[8] This study also showed that macrophages were the cell type predominantly involved in its production and that the enhanced production occurred on a 'per cell' basis. This is likely to be due to the increased proportion of activated macrophages in ulcerative colitis and Crohn's disease.[13][14]

In this study, we have examined the presence of this cytokine in biopsy specimens from normal colonic mucosa and from colons with active inflammatory bowel disease. Greater amounts of interleukin 1β were present in the tissue from inflamed colons. Organ culture of biopsy specimen over 24 hours suggested that synthesis of this cytokine occurs, although the amount synthesised may be an underestimate as metabolism of the peptide may also be occurring in culture.

After 24 hours' culture, greater amounts of interleukin 1β were detected in cultures of inflamed biopsy specimens. The amount of interleukin 1β present in the mucosa was dependent on the presence and degree of inflammation and not on the type of treatment received by the patient.

5-Aminosalicylic acid, which is the active moiety of sulphasalazine,[15] reduced the amount of interleukin 1β detected after culture. This occurred at concentrations of the drug well below those detected in the therapeutically relevant, free faecal water in the colonic lumen of patients taking sulphasalazine.[16] In contrast, sulphapyridine, which is the 'carrier' or inactive drug in sulphasalazine, did not affect interleukin 1β production in culture in concentrations

similar to those for 5-aminosalicylic acid. Dexamethasone has previously been shown to inhibit synthesis of interleukin 1β by peripheral blood monocytes by poststranscriptional mechanism[9] and our study has shown that inhibition also occurs in inflamed rectal mucosa.

We do not believe that the effects of 5-aminosalicylic acid and dexamethasone on the synthesis of interleukin 1β were due to an effect on the viability of the cells in organ culture. The inhibition observed was specific to these drugs and not seen with sulphapyridine. Histological studies after culture showed excellent preservation of tissue which was unaffected by the presence of the drugs. Culture of isolated peripheral blood mononuclear cells in the presence of 5-aminosalicylic acid, dexamethasone, or sulphapyridine, in the concentrations used above, did not affect the viability of these cells. There was also no significant difference in the amount of thromboxane synthesised in the presence of 5-aminosalicylic acid. Thus, a separate synthetic function of the cells in the biopsy specimens was not affected suggesting that 5-aminosalicylic acid has a specific effect on interleukin 1β synthesis rather than affecting the viability of cells in the biopsy specimens.

The mechanism by which 5-aminosalicylic acid may affect production of interleukin 1β by biopsy tissues in organ culture is unknown. Previous studies have shown that synthesis of prostaglandin E_2 and lipoxygenase products is enhanced with inflammation[17-20] and that 5-aminosalicylic acid can inhibit synthesis of both prostaglandin E_2 and 5 lipoxygenase products including leukotriene B_4.[17 20-23] Prostaglandin has been shown to inhibit synthesis of interleukin 1[24] whereas cyclooxygenase inhibitors and leukotrienes enhance its production.[25] In our study, 5-aminosalicylic acid inhibited synthesis of leukotriene B_4 by inflamed colonic biopsy tissues in organ culture. An effect on basal synthesis of leukotriene B_4 has not been shown before, as previous studies have investigated short term leukotriene B_4 synthesis by biopsy specimens stimulated with ionophore.[20 26]

Significantly greater amounts of thromboxane B_2 were synthesised by inflamed biopsy tissues in organ culture. Sulphasalazine has previously been found to be a potent inhibitor of thromboxane B_2 synthesis[22 27] and less potent inhibition by 5-aminosalicylic acid has also been shown.[27] At the concentrations used in this study, 5-aminosalicylic acid did not affect thromboxane B_2 synthesis by inflamed biopsy tissues in organ culture. Values of thromboxane B_2 in medium of organ culture of biopsy specimens correlated with those of interleukin 1β and we wondered whether thromboxane B_2 might be involved in production of interleukin 1β. However, the inhibition of interleukin 1β by 5-aminosalicylic acid occurred without changes in thromboxane B_2 values and therefore probably involves a thromboxane independent mechanism. There were not enough samples to compare values of leukotriene B_4 in medium of organ cultures of normal and inflamed biopsy specimens.

Our study leaves unclear the question of whether 5-aminosalicylic acid affects interleukin 1β synthesis by altering synthesis of other eicosanoids such as prostaglandin E_2 of leukotriene B_4. Since prostaglandin E_2 and leukotriene B_4 have opposite effects on interleukin 1 production and synthesis of both can be inhibited by 5-aminosalicylic acid, it is possible that the drug has a direct effect on the process of gene transcription or translation.

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