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Brief Report   Oncology

Allergic contact dermatitis (ACD) is well recognized as an adverse event associated with implantable medical devices that contain allergenic materials like nickel; however, other cutaneous consequences of chronic exposure to allergens in implanted devices are not well understood. Here, we present a clinical case of Marjolin's ulcer, an invasive squamous cell carcinoma (SCC) that developed in response to chronic ACD caused by an orthopedic implant. We used a standard murine model of contact hypersensitivity to determine whether chronic ACD promotes skin carcinogenesis. Chronic application of 1-fluoro-2,4-dinitrobenzene (DNFB) to carcinogen-treated skin led to the development of papillomas and aggressive SCC. DNFB-driven chronic ACD was marked by type 2 inflammation, which mediated skin carcinogenesis, as mice unable to mount an inflammatory response were less likely to develop skin tumors. Importantly, we found similar tumor-promoting inflammation surrounding the SCC in our patient. Our findings demonstrate that chronic ACD caused by constant exposure to an allergen can promote tumorigenesis at skin sites with preexisting cancer-initiated cells. Moreover, our results suggest that patients with implantable devices placed in close proximity to the skin should be monitored for ACD and highlight the importance of patch testing prior to the placement of such devices.

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Allergic contact dermatitis (ACD) is well recognized as an adverse event associated with implantable medical devices that contain allergenic materials like nickel; however, other cutaneous consequences of chronic exposure to allergens in implanted devices are not well understood. Here, we present a clinical case of Marjolin's ulcer, an invasive squamous cell carcinoma (SCC) that developed in response to chronic ACD caused by an orthopedic implant. We used a standard murine model of contact hypersensitivity to determine whether chronic ACD promotes skin carcinogenesis. Chronic application of 1-fluoro-2,4-dinitrobenzene (DNFB) to carcinogen-treated skin led to the development of papillomas and aggressive SCC. DNFB-driven chronic ACD was marked by type 2 inflammation, which mediated skin carcinogenesis, as mice unable to mount an inflammatory response were less likely to develop skin tumors. Importantly, we found similar tumor-promoting inflammation surrounding the SCC in our patient. Our findings demonstrate that chronic ACD caused by constant exposure to an allergen can promote tumorigenesis at skin sites with preexisting cancer-initiated cells. Moreover, our results suggest that patients with implantable devices placed in close proximity to the skin should be monitored for ACD and highlight the importance of patch testing prior to the placement of such devices.

Introduction

Implantable devices like orthopedic, dental, and cardiac implants are an increasingly indispensable part of medical therapeutics. With regard to orthopedic devices, over 1 million lower-extremity joint replacements are performed each year in the United States, and this number is predicted to rise in the future due to the aging population, increase in the rate of obesity, and expansion of medical indications (1). These devices contain metal alloys including nickel, cobalt, and chromium that provide their strength and long-term durability. However, allergy to metals included in the implants is commonly seen, with about 20% of the US population being allergic to nickel (2). Chronic inflammation from metal joint replacements may cause complications at the implant site, which mostly present as chronic joint pain, swelling, loosening, and joint failure (3). For other implants placed in close proximity to the skin, allergic contact dermatitis (ACD) can ensue in 0.1% to 5% of metal implant cases (4, 5). The mechanism of acute ACD is well understood and involves a type IV hypersensitivity reaction mediated by Th1 cells responding to hapten-modified proteins (6), while chronic ACD results in Th2-polarized inflammation (7, 8). The long-term consequences of chronic ACD caused by allergenic metal implants are poorly understood.

Here, we present a clinical case of Marjolin's ulcer, an invasive squamous cell carcinoma (SCC) that developed in the context of nickel allergy from a subcutaneous metal implant. Using animal models, we demonstrate that prolonged ACD markedly enhances the development of skin cancer at the skin site exposed to a carcin-ogen. Further, we show that chronic ACD has the cardinal features of a protumorigenic inflammatory environment and is responsible for this tumor promotion.

Results and Discussion

A 46-year-old white female with no history of skin cancer had an ankle fracture 3 years earlier, which was repaired with open reduction and internal fixation with metal rod placement on the lateral aspect of her fibula for stabilization. After the initial surgery, the patient developed a nonhealing skin lesion on her left ankle overlying the metal implant and the surgical wound site. Subsequently, she was found to be allergic to the nickel in the metal implant (Figure 1A). The implant was removed a year after the initial surgery, but the skin lesion persisted. When the patient presented to us with a 3-year history of the ulcerated skin lesion, she had significant erythema, pain, and oozing at the lesion site and evidence of chronic sun exposure in the nonlesional skin (Figure 1B). A biopsy showed a well-differentiated but invasive SCC consistent with a Marjolin's ulcer (Figure 1C). Due to the depth of invasion and concern for metastasis, the patient underwent a PET-CT scan, which was negative (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI77843DS1). The SCC was surgically excised with a clear margin without signs of recurrence over a 2-year follow-up period.

The development of a high-risk SCC in an individual without a history of skin cancer strongly suggested that the chronic ACD surrounding the surgical wound on the patient's sun-exposed skin culminated in cancer formation. To determine whether chronic ACD can synergize with the surgical wound to promote skin tumorigenesis, we examined the tumor-promoting potential of contact hypersensitivity in mice. Due to species-specific
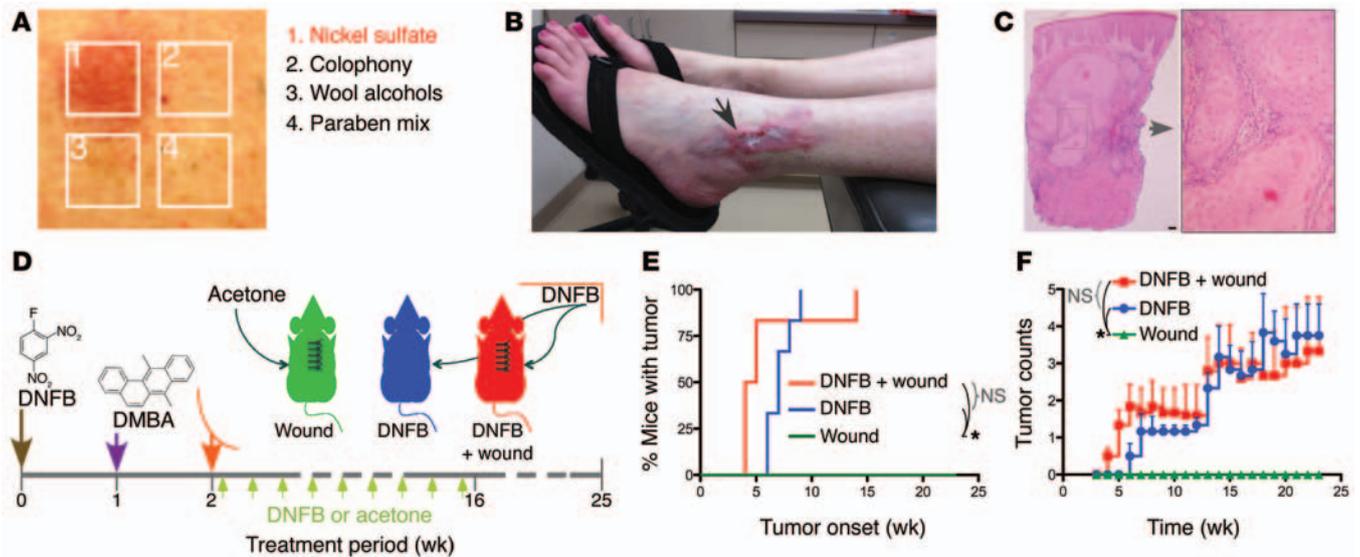
Figure 1. Chronic ACD promotes skin tumorigenesis. (**A**–**C**) Clinical case of an invasive SCC that developed in the context of ACD to nickel is shown. (**A**) Skin-patch test result indicated nickel allergy. (**B**) SCC lesion on the patient's left lateral ankle. Note the solar lentigines on the patient's leg that are a sign of chronic sun exposure. Also, there were no signs of vascular compromise or stasis changes (arrow marks the biopsy site). (**C**) Punch biopsy of the lesion demonstrated a well-differentiated, but invasive, SCC (scale bar: 100 μm), consistent with Marjolin's ulcer. (**D**) Experimental design used to investigate whether chronic ACD synergizes with the surgical wound to promote tumor development. (**E**) Time to tumor onset and (**F**) average number of tumors per animal for each group. Regardless of the presence of a surgical wound, animals chronically treated with DNFB developed skin tumors with short latency (*$P < 0.001$ by log-rank test) and increasing tumor counts over time (*$P < 0.05$ starting from week 7 by Student's t test). No skin tumors developed in the acetone-treated group, despite carcinogen exposure. Age-matched FVB female mice were used in this study; $n = 6$ per group.

susceptibility, mice do not respond to nickel, and we therefore studied hapten-induced contact hypersensitivity, which induces similar inflammatory responses (Figure 1D and refs. 9, 10). First, we exposed a group of age-matched female FVB mice to acetone (carrier) or 1-fluoro-2,4-dinitrobenzene (DNFB) on their abdomen at day 0. One week later, their back skin was treated with a single dose of 7,12-dimethylbenz(a)anthracene (DMBA), a commonly used carcinogen that mimics the cancer-initiating effect of sun exposure (11–13). The following week, the animals were randomized for surgical wounding and placed into 1 of the following 3 groups: (a) acetone (carrier) treatment plus surgical wounding; (b) DNFB treatment; or (c) DNFB treatment plus surgical wounding. After surgical incision, suture placement, and the first topical treatment, the animals were treated every 2 to 3 days with DNFB or acetone for 14 weeks and followed for an additional 9 weeks (Figure 1D). Animals receiving DNFB developed skin tumors with a short latency, while none of the animals treated with acetone alone developed tumors, despite the carcinogen ($P < 0.01$, Figure 1, E and F). Notably, the tumor-promoting effect of the allergen was dominant and masked any contribution from a surgical wound. In addition, skin tumors that formed during the allergen treatment period persisted after treatments were stopped (Figure 1F). Although the DNFB-treated animals each developed a few skin tumors (Figure 1F), they experienced a high rate of tumor progression from papilloma to SCC, leading to their early demise ($P < 0.05$; Supplemental Figure 2A). The SCCs were well-to-moderately differentiated invasive carcinomas with aggressive behavior reminiscent of the SCC in our clinical patient (Supplemental Figure 2B). Thus, chronic ACD markedly enhanced tumor formation.

To determine whether DNFB-induced chronic ACD results in a tumor-promoting environment, we analyzed the dorsal skin of animals treated with DNFB versus acetone at the end of the chronic allergen treatment period (Figure 1D). The DNFB-treated skin showed marked epidermal hyperplasia, with dense dermal inflammation compared with that of the acetone-treated controls (Figure 2A). Chronic ACD in our animals was associated with a Th2-dominant immune response, characterized by abundant production of $Il4$ but not $Ifng$ (Figure 2B and refs. 14, 15). This is consistent with previous studies demonstrating the switch from type 1 inflammation in acute ACD to type 2 in the chronic phase (7, 8). In addition, the DNFB-treated skin expressed significantly higher levels of $Il6$ compared with levels detected in the acetone-treated controls (Figure 2C and ref. 16). In addition to CD4+ T cells, the number of mast cells, alternatively activated (M2) macrophages (F4/80+CD206+), and blood vessels was markedly increased upon chronic DNFB treatment (Figure 2D and Supplemental Figure 3). Each of these factors has been implicated in tumor promotion (14, 17, 18). Therefore, chronic ACD results in the critical elements of a tumor-promoting inflammatory environment in the skin.

To confirm that the tumor-promoting effect of DNFB is driven by chronic ACD and not by a direct effect of the chemical on keratinocytes (tissue source of the tumor) (19), we studied $Rag1^{-/-}$ (Rag-KO) and $Rag2^{-/-}$, $\gamma c^{-/-}$ (Ragγc-KO) animals (Figure 1D) that lack adaptive and innate immune cells implicated in the induction of ACD (20, 21). Although WT, Rag-KO, and Ragγc-KO animals showed a similar degree of hair loss and skin irritation in response to DNFB (Supplemental Figure 4), Rag-KO and Ragγc-KO animals developed significantly less inflammation in response to chronic DNFB treatment (Supplemental Figure 5A). Likewise, Rag-KO
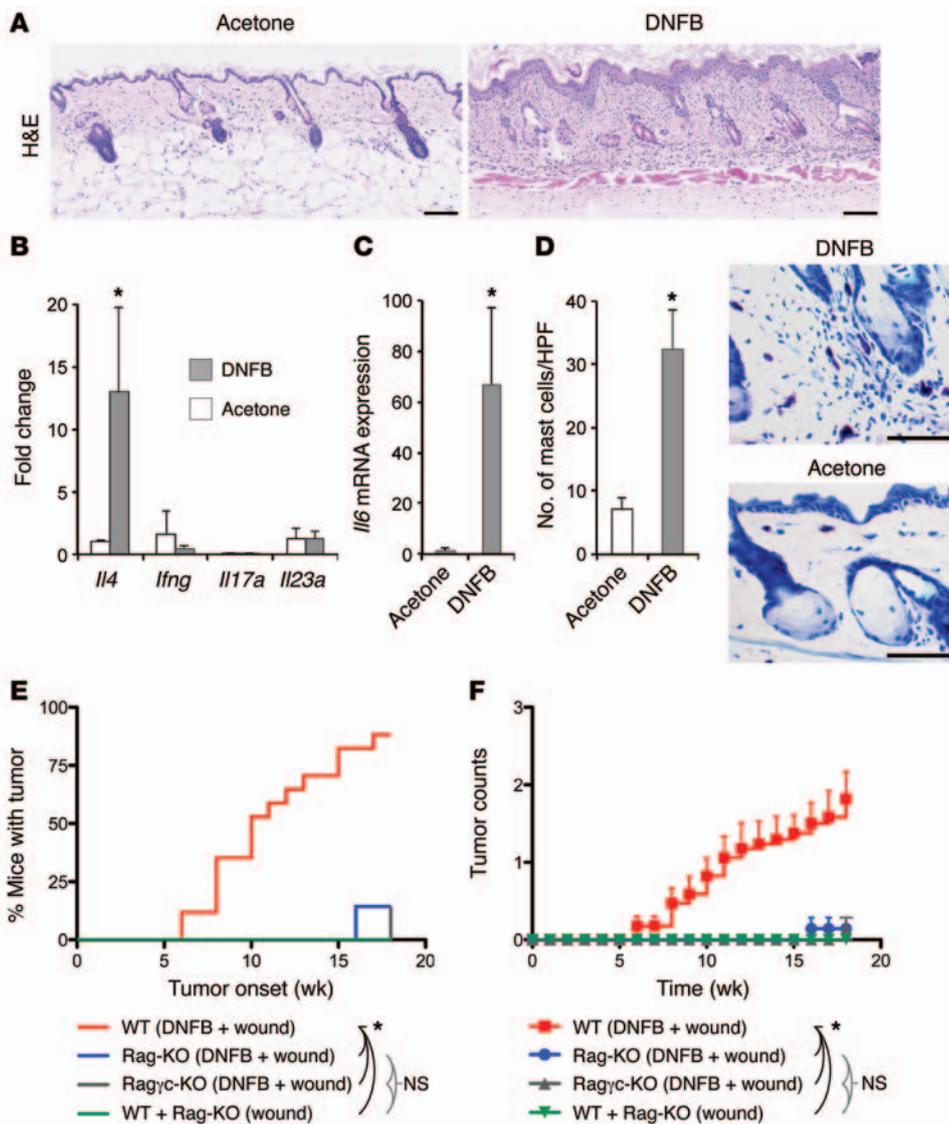
Figure 2. Chronic ACD manifests protumorigenic factors and mediates the tumor-promoting effects of DNFB. (**A**) Representative H&E-stained skin images from mice treated with DNFB for 14 weeks and their acetone-treated controls (scale bars: 100 μm). Note the extent of epidermal hyperplasia and dermal inflammation in the DNFB-treated skin. (**B**) Quantitative real-time PCR analysis of cytokine expression by major Th cell subtypes performed on skin from mice treated with DNFB for 14 weeks compared with cytokine expression in acetone-treated controls ($n = 4$ in each group; $*P < 0.05$ by Student's t test). (**C**) *Il6* expression in skin treated with DNFB for 14 weeks was compared with that in skin of acetone-treated controls ($n = 4$ in each group; $*P < 0.05$ by Student's t test). (**D**) Average number of mast cells in 6 random high-power microscopic fields (HPF) of skin from animals treated chronically with DNFB was compared with that of acetone-treated mice ($*P < 0.05$ by Student's t test). Representative images of toluidine blue–stained skin sections show increased number, size, and granularity of mast cells in DNFB-treated skin (scale bar: 50 μm). (**E** and **F**) Tumor outcome of Rag-KO, Ragγ-KO, and WT animals in response to DNFB plus surgical wounding (following the protocol described in Figure 1D). (**E**) Shown are the time to tumor onset ($*P < 0.01$ by log-rank test) and (**F**) average number of tumors per animal for each of the 4 groups ($*P < 0.05$ starting from week 10 by Student's t test). Age-matched C57BL/6 female mice were used in this study; $n > 7$ per group.

and Ragγ-KO animals treated with DNFB failed to develop any significant epidermal hyperplasia, accumulation of mast cells, M2 macrophages, or blood vessels in their skin compared with what we observed in the DNFB-treated WT animals (Supplemental Figure 5). Importantly, Rag-KO and Ragγ-KO animals treated with DNFB and surgical wounding showed a significantly lower propensity for skin tumor development compared with their age-matched WT animals on a C57BL/6 genetic background ($P < 0.01$, Figure 2, E and F). These findings demonstrate that chronic exposure to a hapten-producing allergen induces tumor-promoting inflammation in the skin.

Finally, we examined the immune environment of the SCC that developed in our clinical case. A dense dermal inflammation was present, surrounding the focus of skin cancer, which was dominated by Th2 cells with characteristic expression of GATA3 but not T-bet (Figure 3A). In addition, the other cellular hallmarks of the tumor-promoting chronic ACD environment were present in the patient's lesional skin, as shown by a marked increase in blood vessels, M2 macrophages, and mast cells (Figure 3, B and C, and Supplemental Figures 6 and 7). Importantly, the inflammation was not

confined to the local environment of the SCC and extended with similar intensity into the cancer-free margins of the excision (Figure 3C and Supplemental Figure 7). These features strongly suggest that the patient's inflammation was the driver of the carcinogenesis and not secondary or reactive to cancer development itself.

Our findings provide clear evidence for the tumor-promoting property of chronic ACD. Chronic ACD can thus lead to the development of Marjolin's ulcer–like invasive SCC in areas where a significant burden of cancer-initiated cells is present (i.e., sun- or carcinogen-exposed skin). The chronic nature of ACD caused by implantable devices that are placed adjacent to sun-exposed skin can thus provide this "perfect storm," as seen in our clinical patient. Although rare, the morbidity and mortality associated with such aggressive cancers highlight the importance of skin-patch testing prior to the placement of implantable devices, especially in patients with known metal allergies (22).

Interestingly, skin cancer development in the context of metal allergy has also been observed with metal dental restorations (23, 24). In addition, there are multiple clinical case reports of SCC development in tattoos, and ACD due to metals used in tattoo dyes
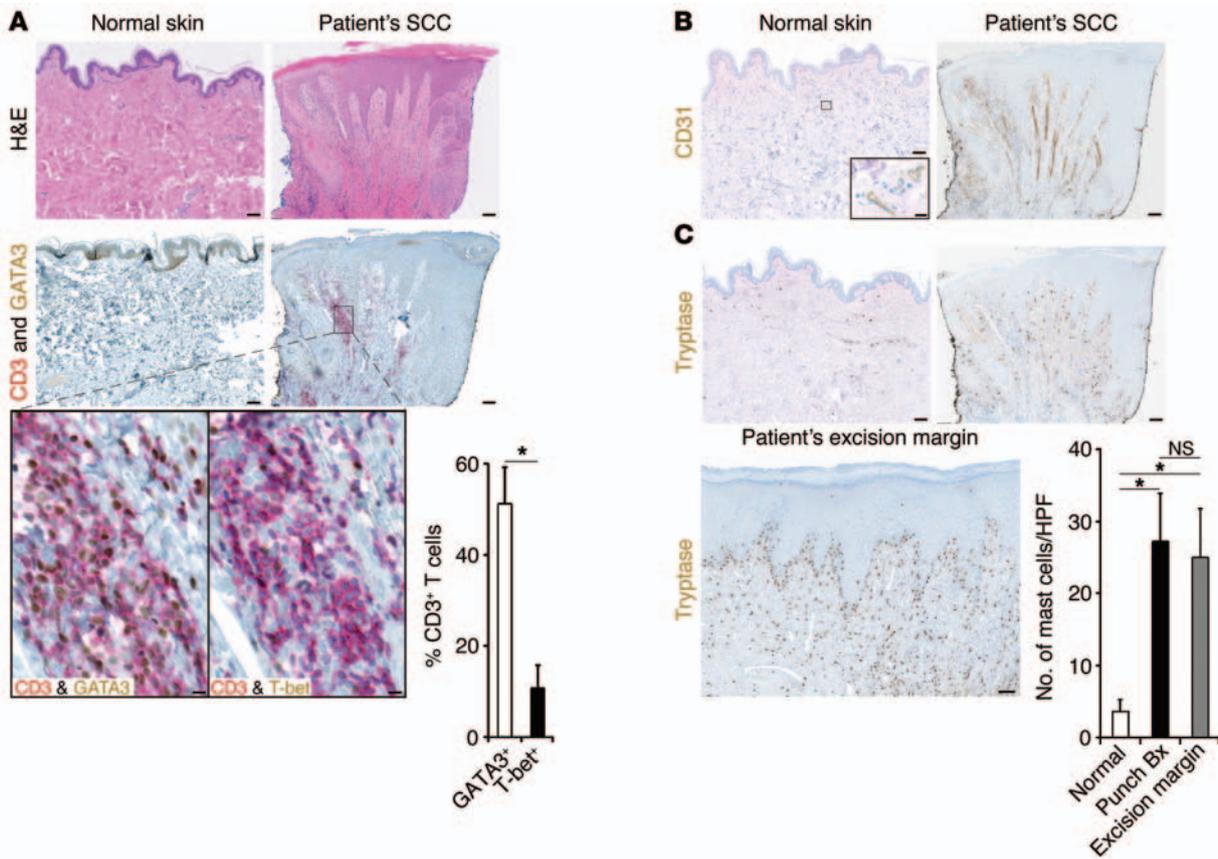
Figure 3. Tumor-promoting inflammation is associated with patient's SCC. (**A**) H&E staining of the punch biopsy specimen highlights the extent of inflammation associated with SCC compared with that in normal skin. CD3, GATA3, and T-bet staining of the patient's SCC shows significant accumulation of GATA3+ T cells (i.e., Th2 cells) in the dermis surrounding the SCC (quantified as the percentage of CD3+ cells counted in 6 random HPFs). (**B**) CD31 staining marks the vascular density surrounding the patient's SCC compared with that in the normal skin. (**C**) Average number of mast cells in 6 random HPFs near the site of SCC (Punch Bx) and beyond at the excision margins was compared with the number of mast cells in the normal skin. The increased number of mast cells in the excision margins indicates that the patient's skin inflammation extended beyond the cancer site. Representative images are shown. *$P < 0.05$. Scale bars: 100 μm; 10 μm (enlarged images in **A** and inset in **B**).

has been reported to precede the development of such cancers (25, 26). Our findings provide a mechanistic explanation for skin cancer development in these clinical contexts. Taken together, our study describes the mechanism of a severe adverse event associated with implantable materials and calls for close monitoring of patients receiving these implants to avoid chronic ACD and the possibility of developing SCC.

Methods

Patient samples. The initial punch biopsy of the SCC and the completely excised lesion with clear margins were fixed in formalin and embedded in paraffin. H&E staining was performed using Tissue-Tek Prisma Stainer (Sakura Finetek USA). Formalin-fixed, paraffin-embedded tissue sections (4-μm) were immunostained using a Ventana ULTRA automated immunostainer (Ventana Medical Systems).

Statistics. To test the significance between study groups, we used a log-rank test for tumor onset and survival data and a 2-tailed Student's *t* test for tumor counts and other quantitative measurements. A *P* value of less than 0.05 was considered statistically significant. All bar graphs show the mean + SD.

Study approval. The clinical study was approved by the IRB of Washington University, and written informed consent was obtained to

publish the patient's photographs and study her skin cancer. All animal studies were approved by the IACUC of Washington University.

Refer to the Supplemental Methods for a further description of the methods.

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