Inhibition of ADP Ribosylation Prevents and Cures Helicobacter-Induced Gastric Preneoplasia

Isabella M. Toller¹, Matthias Altmeyer², Esther Kohler¹, Michael O. Hottiger², and Anne Müller³

Abstract
Gastric adenocarcinoma develops as a consequence of chronic inflammation of the stomach lining that is caused by persistent infection with the bacterium Helicobacter pylori. Gastric carcinogenesis progresses through a sequence of preneoplastic lesions that manifest histologically as atrophic gastritis, intestinal metaplasia, and dysplasia. We show here in several preclinical models of Helicobacter-induced atrophic gastritis, epithelial hyperplasia, and metaplasia that the inhibition of ADP ribosylation by the small-molecule inhibitor PJ34 not only prevents the formation of gastric cancer precursor lesions, but also efficiently reverses preexisting lesions. PJ34 exerts its chemopreventive and therapeutic effects by impairing Helicobacter-specific T-cell priming and T₃₁ polarization in the gut-draining mesenteric lymph nodes. The subsequent infiltration of pathogenic T cells into the gastric mucosa and the ensuing gastric T-cell–driven immunopathology are prevented efficiently by PJ34. Our data indicate that PJ34 directly suppresses T-cell effector functions by blocking the IFN-γ production of mesenteric lymph node T cells ex vivo. Upon exposure to PJ34, purified T cells failed to synthesize ADP-ribose polymers and to activate the transcription of genes encoding IFN-γ, interleukin 2, and the interleukin 2 receptor α chain in response to stimuli such as CD3/CD28 cross-linking or phorbol 12-myristate 13-acetate/ionomycin. The immunosuppressive and chemoprotective effects of PJ34 therefore result from impaired T-cell activation and T₃₁ polarization, and lead to the protection from preneoplastic gastric immunopathology. In conclusion, ADP-ribosylating enzymes constitute novel targets for the treatment of Helicobacter-associated gastric lesions predisposing infected individuals to gastric cancer and may also hold promise for the treatment of other T cell–driven chronic inflammatory conditions and autoimmune pathologies. Cancer Res; 70(14); 5912–22. ©2010 AACR.

Introduction
Epidemiologic and experimental studies have established unequivocally that chronic inflammatory diseases predispose to cancers of the liver, cervix, colon, stomach, and several other organs (1). In the stomach, chronic inflammation caused by persistent infection with the Gram-negative bacterial pathogen Helicobacter pylori is the most important risk factor for the development of adenocarcinoma (2–5). In a high-risk subset of infected individuals, H. pylori causes multifocal corpus-predominant atrophic gastritis, which frequently progresses to intestinal metaplasia, occasionally to dysplasia, and rarely to adenocarcinoma (6, 7). We have shown recently using a C57BL/6 mouse model of Helicobacter infection that the early stages of this preneoplastic sequence develop as a result of gastric infiltration by T₃₁-polarized, MHC class II–restricted effector T cells (8). This T-cell subset is, on the one hand, absolutely essential for the efficient control of infection and, on the other hand either directly or indirectly induces gastric epithelial transformation (8). Both phenotypes depend on IFN-γ, which is secreted by T₃₁-polarized effector T cells both locally at the site of infection and in the gut-draining mesenteric lymph nodes (MLN). Targeting T cells pharmacologically in the infectious process prevents and even reverses established premalignant lesions, confirming that T cells are crucial mediators of gastric preneoplasia (9).

Poly(ADP-ribose) (PAR) is synthesized by the PAR polymerase (PARP) family of enzymes, which use the substrate NAD⁺ to ADP-ribosylate acceptor proteins (10–12). PARP enzymes have been implicated in the pathogenesis of several chronic inflammatory and autoimmune conditions, and NAD⁺-mimicking inhibitors of poly(ADP-ribosyl)ation efficiently block inflammation in rodent models of Crohn’s disease (13) and in allergen-induced airway inflammation models (14), and protect mice from endotoxic shock (15). Since the concept of synthetic lethality of PARP inhibitors in cells with defects in homologous recombination was
introduced in 2005 (16, 17), PARP inhibitors have entered the arena of cancer therapy, showing a benefit in breast and ovarian cancer patients with BRCA1- or BRCA2-deficient tumors (18–21).

We show here that the T cell–driven gastric preneoplastic immunopathology observed in Helicobacter–infected C57BL/6 mice is attenuated by systemic administration of PJ34, a NAD+ analogue and inhibitor of ADP ribosylation originally designed to inhibit PARP1 (22). The same treatment further efficiently reversed preexisting lesions, especially when applied in combination with Helicobacter eradication therapy. We find that PJ34 exerts its chemopreventive and therapeutic effects by inhibiting the priming and programming of Th1 polarized T cells in the gut-draining lymph nodes. An essential role for ADP ribosylation during T-cell activation was shown with CD3/CD28 cross-linking were blocked by PJ34. In conclusion, we propose that ADP-ribosylating enzymes in T cells may represent novel targets for the treatment of gastric premalignant lesions in patients that are refractory to Helicobacter eradication therapy.

**Materials and Methods**

**Animal experimentation, Helicobacter strains, and pharmacologic treatments**

Male C57BL/6, interleukin (IL)-10−/−BL/6, CD4−/−BL/6, and MyD88−/−BL/6 mice (Charles River Laboratories) as well as PARP1−/−BL/6 (23) and PARP2−/−BL/6 (a kind gift from Gilbert de Murcia, Intégrité du Génome, Unité Mixte de Recherche 7175, Ecole Supérieure de Biotechnologie de Strasbourg, Illkirch, France) were maintained in individually ventilated cages and infected at 5 to 6 weeks of age with two consecutive doses of ∼106 *H. felis* CS1 (ATCC 49179; ref. 24). All animal experiments were approved by the cantonal veterinary office. For in vivo inhibition of ADP ribosylation, PJ34 [N-(6-Oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamid, Enzo Life Sciences] was administered in drinking water supplemented with 0.1 mg/mL Aspartame, resulting in a daily dose of 30 mg/kg of body weight. In cell culture experiments, PJ34 and DAM-TIQ-A [2-Dimethylaminomethyl-4H-thieno (2,3-c)isoquinolin-5-one, Enzo Life Sciences] were each used at 15 μmol/L. Antibiotic eradication therapy was achieved by 2 weeks of daily orogastric treatment with 4.5 mg/mL metronidazole, 10 mg/mL tetracycline hydrochloride (both Sigma-Aldrich), and 1.2 mg/mL bismuth subcitrate (Park-Davis).

**Assessment of *H. felis* colonization, gastric IFN-γ production, gastric neutrophil and lymphocytic infiltration, and histopathology**

Murine stomachs were dissected longitudinally into equally sized pieces. For the assessment of *H. felis* colonization, total genomic stomach DNA was subjected to quantitative PCR analysis of the flaB gene, as previously described (8). The quantitative analysis of gastric IFN-γ expression by real-time reverse transcription-PCR (RT-PCR) and the quantification of neutrophil infiltration by myeloperoxidase activity assay were performed as described (8). Gastric lymphocyte single-cell suspensions were generated by digestion with 0.25 mg/mL collagenase IV (Sigma) and passing through a cell strainer. PARP1 expression was analyzed by immunoblotting of mucosal extract using rabbit anti-PARP1 antibody H-250 (Santa Cruz). Gastric histopathology was graded on Giemsa-stained paraffin sections as described in detail previously (8, 9). All pictures were taken with a Leica Leitz DM RB microscope equipped with a Leica DFC 420C camera. Images were acquired at ×20 magnification using the Leica Application Suite 3.3.0 software. Scale bars indicate 50 μm. The immunomagnetic isolation of lymphocytes from MLN and spleen, and the assessment of lymphocyte proliferation as well as IFN-γ and IL-2 expression by real-time RT-PCR and intracellular staining is described in the Supplementary Methods.

**Statistical analysis**

*P* values were calculated using GraphPad prism 5.0 software. The significance of differences in histopathology categories was calculated by Mann-Whitney test, and the significance of numerical differences was calculated by Student’s *t* test. All *in vitro* assays were analyzed in triplicate and are shown with SDs.

**Results**

**Inhibition of ADP ribosylation by PJ34 in vivo attenuates Helicobacter-induced atrophic gastritis**

As ADP-ribosylating enzymes have been implicated in chronic inflammatory and autoimmune disorders, and also in the development and progression of certain neoplasms (13, 14, 16, 17), we hypothesized that blocking ADP ribosylation *in vivo* would modulate *Helicobacter*-induced gastric (pre) neoplasia. To validate the use of the NAD+ analogue PJ34, an inhibitor of PARPs and of ADP ribosylation in general, we first used the standardized human AGS gastric epithelial cell culture model of *H. pylori* infection. PAR formation could be detected in *H. pylori*-infected AGS cells as early as 6 hours postinfection (Supplementary Fig. S1A). The synthesis of PAR was completely blocked by the addition of PJ34 (Supplementary Fig. S1B), implying that PJ34 readily permeates into cultured cells and efficiently blocks all enzymes involved in *Helicobacter*-dependent PAR formation.

Experimental infection of C57BL/6 mice with the close *H. pylori* relative *H. felis* mimics the human host’s response to the bacterium, with gastric lesions manifesting histologically as chronic atrophic gastritis accompanied by epithelial hyperplasia and acidic mucus–positive metaplasia (8, 9, 25, 26). The formation of preneoplastic lesions is strongly accelerated in IL-10−/− mice, which develop atrophy, hyperplasia, and metaplasia as early as 4 weeks postinfection (Fig. 1A) compared with ∼12 weeks postinfection in wild-type (WT) mice (8). IL-10−/− mice thus constitute a convenient model for pharmacologic intervention.

IL-10−/− mice were given PJ34 with the drinking water at a daily dose of 30 mg/kg of body weight. The quantitative analysis of gastric histopathology revealed that PJ34 treatment strongly reduced *Helicobacter*-associated inflammation,
atrophy, and epithelial hyperplasia and, to a lesser extent, intestinal metaplasia (Fig. 1A). In agreement with the effects of PJ34 on gastric histopathology, the mucosal infiltration of neutrophils as determined by myeloperoxidase activity assay was reduced in the presence of the inhibitor (Fig. 1B). PJ34 treatment further significantly impaired the clearance of Helicobacter that is typically observed in IL-10−/− mice (Fig. 1C). To clarify whether PJ34 exerts its protective effects through inhibition of PARP1, the target for which PJ34 was originally designed, we first analyzed PARP1 expression in the gastric mucosa of infected and uninfected IL-10−/− mice (Fig. 1D). All infected mice showed higher levels of PARP1 than their age-matched uninfected counterparts; PARP1 levels were not influenced by PJ34 (Fig. 1D). To investigate the functional consequences of Helicobacter-associated PARP1 accumula-
tion in the gastric mucosa, we crossed IL-10−/− mice with PARP1−/− mice to generate IL-10−/−/PARP1−/− animals. We assessed the development of gastritis and preneoplastic lesions in this strain over time compared with the PARP1-proficient parental strain. Remarkably, no significant differences could be observed with respect to the onset or the severity of lesions (5, 14, and 21 d postinfection; Supplementary Fig. S2), implying that PJ34 acts through mechanisms not dependent only on PARP1 to attenuate Helicobacter-associated gastric pathology in vivo.

Inhibition of ADP ribosylation by PJ34 reverses preexisting lesions

We next examined a possible therapeutic effect of PJ34; to this end, we had to abandon the IL-10−/− model, as these

Figure 1. Inhibition of ADP ribosylation prevents Helicobacter-induced gastritis and precancerous lesions. IL-10−/− mice were infected for 4 wk with Helicobacter felis or remained uninfected. One infected group received PJ34 (30 mg/kg/d) during the last 2 wk of infection. A, a schematic treatment overview, representative Giemsa-stained sections, and histopathology scores are shown for all mice. Gastric histopathology was assessed with respect to chronic inflammation, atrophy, metaplasia, and compensatory epithelial hyperplasia; scores were assigned on a scale of 0 to 6. Horizontal columns, mean values. Scale bars, 50 μm. B, neutrophil infiltration into the stomach was assessed by myeloperoxidase activity assay; columns, means; bars, SD. C, Helicobacter colonization was analyzed by quantification of the flaB gene copy number by real-time PCR of gastric genomic DNA. Ns, not significant. D, gastric PARP1 expression was analyzed for three to four mice per group by immunoblotting. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels are shown as loading controls.
mice clear the infection (Fig. 1C) and spontaneously revert to normal gastric histology by 6 to 8 weeks postinfection (data not shown). For therapeutic studies, we used CD4−/− mice that develop premalignant lesions more consistently than C57BL/6 WT mice by 3 months postinfection, creating a therapeutic window for pharmacologic intervention between 3 and 4 months postinfection (Fig. 2). The increased susceptibility of CD4−/− mice is presumably due to their lack of a CD4+ regulatory T-cell population that could control the immunopathology induced by "pathogenic" effector T cells, which are CD8+ in the absence of CD4+ T cells (27).

CD4−/− mice were infected with H. felis for 3 months, and the development of premalignant pathology was confirmed in a small control group (data not shown). The remaining mice either received PJ34 in the 4th month postinfection or remained nontreated. The treatment with PJ34 efficiently reduced all parameters of gastric histopathology (Fig. 2A). The regression of lesions was accompanied by a significant reduction of gastric IFN-γ production and neutrophil infiltration (Fig. 2B and C). In line with our previous observations (8, 9), the reduction of IFN-γ and mucosal neutrophils was associated with an increase in Helicobacter colonization (Fig. 2D), which is indicative of a reversal to normal gastric mucosal architecture and physiology.

**The regression of preneoplastic lesions is not sustained under conditions of ongoing Helicobacter colonization**

To confirm these results in an independent model, we took advantage of a mouse strain that lacks the gene encoding the adaptor protein MyD88. MyD88−/− mice develop severe preneoplastic lesions as early as 1 month postinfection (Supplementary Fig. S3A), presumably due to a defect in immune counter-regulation.6 However, in contrast to CD4−/− and IL-10−/− mice, which efficiently reduce H. felis colonization or even clear the infection, respectively (Figs. 1 and 2), MyD88−/−

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6 A. Sayi, I. Toller, A. Mueller, unpublished observations.
mice are colonized heavily for at least 3 months postinfection (Fig. 3). We used the therapeutic window between 1 and 3 months postinfection to test whether the reversal of preneoplastic lesions by PJ34 treatment would be sustained after termination of the treatment, despite ongoing \textit{H. felis} colonization, or would require the concomitant eradication of the infection (Fig. 3).

\textit{MyD88}^{-/-} mice were infected for 1 month, and the development of pathology was verified in a small control group (Supplementary Fig. S3A and D). The remaining mice either received PJ34 for the subsequent month, were subjected to eradication therapy in weeks 5 and 6 postinfection, or received both treatments. At 2 months postinfection, the reversal of preexisting lesions and the efficiency of eradication therapy were verified in additional control groups (Supplementary Fig. S3B and D). All remaining mice were maintained for a 3rd month and then analyzed with respect to their colonization and histopathology. Mice that had been continuously colonized throughout the 3-month time course had all developed severe pathology (Fig. 3A). The regression

Figure 3. The regression of preneoplastic lesions is stable under conditions of simultaneous PJ34 treatment and \textit{Helicobacter} eradication. \textit{MyD88}^{-/-} mice were infected for 3 mo and received PJ34 in the 2nd month with or without eradication therapy in weeks 5 and 6 postinfection. Additional mice served as infected or uninfected age-matched controls. Control groups were sacrificed after 1, 2, and 3 mo to verify the success of the respective treatments (see Supplementary Fig. S3). A, schematic overview, representative Giemsa-stained sections, and histopathology scores; horizontal columns, means. B, neutrophil infiltration into the gastric mucosa as quantified by myeloperoxidase assay. C, \textit{H. felis} colonization as quantified by real-time PCR of the \textit{flaB} gene.
of preneoplastic lesions induced by PJ34 treatment during the second month was transient, as the mice relapsed once the treatment was discontinued (Fig. 3A). In contrast, mice that had been cured of their infection while receiving PJ34 showed sustained regression of their lesions, i.e., the effect of the treatment was stable in this group (Fig. 3A). As reported previously (28), eradication therapy by itself was also effective at reversing existing lesions (Supplementary Fig. S3C and D), indicating that Helicobacter eradication and the inhibition of ADP ribosylation have comparable effects in mice. In contrast, eradication therapy typically fails to cure the human counterpart lesions (29), suggesting that the consecutive treatment with antibiotics and ADP ribosylation inhibitors would have a beneficial effect over eradication therapy alone in humans.

The effects on gastric histopathology were reflected in the levels of neutrophil infiltration, which decreased more dramatically in the group that was subjected to both PJ34 treatment and eradication therapy than in the group that received PJ34 only (Fig. 3B). Helicobacter levels were not significantly different between the two infected groups with or without PJ34 treatment; only background levels of Helicobacter-specific PCR products were detectable upon eradication therapy (Fig. 3C). In conclusion, the pharmacologic inhibition of ADP ribosylation for therapeutic purposes is temporarily successful in reversing preexisting lesions, but is sustained only if the underlying infection is eradicated.

Inhibition of ADP ribosylation prevents T-cell priming in MLNs

We have reported previously that gastric IFN-γ responses to Helicobacter infection correlate with the level of gastric T-cell infiltration and preneoplastic pathology (8, 9). This association was again confirmed in this study (Figs. 1 and 2). The proportion of T cells producing IFN-γ (i.e., Th1-polarized effector T cells) in the MLN is a reliable indicator of Helicobacter-specific T-cell responses and a good predictor of gastric pathology (9). To assess whether inhibition of ADP ribosylation modulates gastric histopathology by interfering with T-cell priming, we infected 10 WT C57BL/6 mice with H. felis and treated one half of the group with PJ34 for 1 month. The rest remained nontreated; additional mice served as uninfected, nontreated controls. IFN-γ–positive T cells were quantified in single-cell MLN preparations of individual mice by intracellular staining for IFN-γ combined with surface staining for CD4 (Fig. 4A). The proportion of IFN-γ–cells increased in the CD4+ and CD4− fractions as a result of the infection; this increase was suppressed by PJ34 treatment (Fig. 4A). As a consequence of impaired T-cell priming in the MLN due to PJ34 treatment, fewer leukocytes were present in single-cell preparations of the corresponding stomachs (Fig. 4B, top), and fewer of these gastric leukocytes stained positive for CD4 (Fig. 4B, bottom). To test whether the inhibition of Th1 polarization was due to a direct effect of PJ34 on IFN-γ production, we cultured single-cell preparations from five additional H. felis–infected WT mice for 3 days in the presence or absence of PJ34. All treated cultures revealed a reduction of IFN-γ expression compared with the respective controls (Fig. 4C); the simultaneous visualization of PAR showed that PJ34 indeed significantly reduced PAR formation under these circumstances (Fig. 4C). Finally, we pulsed immunomagnetically isolated MLN-derived dendritic cells with Helicobacter sonicate and cocultured them with splenic CD4+ T cells isolated from uninfected or infected donors (Fig. 4D); the infection-dependent IFN-γ response of the T cells was significantly reduced by PJ34 treatment, as were the background levels of T-cell activation (Fig. 4D). In conclusion, the T-cell priming, Th1 polarization, and IFN-γ production that are a prerequisite for the development of gastric Helicobacter–associated pathology are blocked by PJ34, suggesting that T cells represent the key target of PJ34 in this scenario.

T-cell activation is impaired by inhibition of ADP ribosylation

T-cell activation requires two external signals, an antigen-specific signal that is transmitted through the TCR/CD3 complex and a costimulatory signal received by activation of CD28 (30). Primary T cells are induced to proliferate, to express the activation marker CD25, and to produce and secrete IFN-γ upon CD3/CD28 cross-linking or treatment with phorbol 12-myristate 13-acetate (PMA) and ionomycin (Fig. 5). We isolated splenic CD4+CD25− T cells and stimulated them with anti-CD3/anti-CD28 monoclonal antibody–coated beads or PMA/ionomycin in the presence or absence of either PJ34 or an alternative inhibitor of ADP ribosylation, DAM-TIQ-A. Both treatments induced proliferation of the T cells as assessed by carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution, and proliferation was blocked by both compounds (Fig. 5A). PJ34 was more active than DAM-TIQ-A at the same final concentration. The concomitant upregulation of CD25 was reduced as well (data not shown; Fig. 6B), as was IFN-γ production (Fig. 5C). We conclude that inhibition of ADP ribosylation by either PJ34 or DAM-TIQ-A significantly blocks CD4+CD25− T-cell activation triggered by two alternative stimuli.

To investigate whether the effect of blocking ADP ribosylation was attributable to either PARP1 or PARP2, we stimulated parallel preparations of immunomagnetically isolated WT, PARP1−/−, and PARP2−/− CD4+CD25− T cells by CD3/CD28 cross-linking in the absence or presence of PJ34 and quantified their proliferation (Supplementary Fig. S4). T cells of all three genotypes responded equally well to CD3/CD28 cross-linking: no differences were detected with respect to proliferation (Supplementary Fig. S4), CD25 upregulation, or IFN-γ production (data not shown). Furthermore, we found that PJ34 had similarly suppressive effects on all three genotypes (Supplementary Fig. S4), suggesting that neither PARP1 nor PARP2 are the sole targets of PJ34 in T cells.

To test whether T-cell inhibition by PJ34 is restricted to CD4+ T cells or is a common feature shared by other T-cell subsets, we immunomagnetically isolated splenic CD8+ T cells, subjected them to CD3/CD28 cross-linking, and measured their proliferation (Supplementary Fig. S5). PJ34 treatment indeed blocked the proliferation of CD8+ T cells as efficiently as that of CD4+ T cells, implying that PJ34 has
Figure 4. Inhibition of ADP ribosylation impairs T-cell priming and TH-1 differentiation in the MLNs. A and B, C57BL/6 mice were infected for 1 mo with *H. felis* or remained uninfected; of the infected mice, a subset received PJ34 during the entire course of infection. Single-cell suspensions were generated from the MLN (A) and stomachs (B) of all five mice per group and analyzed individually by flow cytometry. Representative dot plots of CD4 and intracellular IFN-γ signals and averages of all MLN cultures are shown (A). Similarly, representative dot plots and group averages are shown for the percentage of leukocytes and CD4+ T cells per total stomach cells (B, top and bottom). C, additional MLN cultures from five infected mice (numbered 1–5) were cultured for 3 d in the presence or absence of 15 μmol/L PJ34 before intracellular staining for IFN-γ and ADP-ribose polymers.

D, immunomagnetically isolated MLN-derived dendritic cells from uninfected donors were pulsed with Helicobacter sonicate for 16 h and cocultured with splenic CD4+ T cells from uninfected or infected donors before staining of CD4 and intracellular IFN-γ. All events in the CD4+ gate are plotted; representative dot plots and group averages are shown. *P* values were calculated by unpaired (A, B, and D) and paired (C) Student’s *t* test.
broad T-cell immunosuppressive capacity. Interestingly, splenic CD4⁺ T cells isolated from PJ34-treated mice at the time of sacrifice were as responsive to CD3/CD28 cross-linking–induced activation as cells from nontreated donors (data not shown), suggesting that the effects of PJ34 in vivo are transient and do not permanently silence this important branch of the immune system. In conclusion, PJ34 acts as an efficient T-cell immunosuppressant in vitro, suggesting that it may target T cells in a similar manner also in vivo.

**Inhibition of ADP ribosylation blocks il-2 and ifng gene transcription**

Activated T cells require IL-2 for growth (31); therefore, some of the earliest markers of T-cell activation are the up-regulation of the high-affinity α-chain of the IL-2 receptor (CD25; Fig. 5B) and the production of IL-2. IL-2 acts on the producing T cell and its neighbors to sustain their proliferation in an auto and paracrine manner (31). To monitor PAR formation during early stages of T-cell activation upon CD3/CD28 cross-linking, we stained intracellular ADP-ribose polymers after 2, 6, and 16 hours with an antibody that recognizes polymers >16 units in length. PAR formation gradually increased in this time frame and was blocked efficiently by PJ34 (Fig. 6A). Transcription of the il-2 and ifng genes was detectable as early as 2 hours after CD3/CD28 cross-linking (Fig. 6B and C) and remained high after 6 and 16 hours (data not shown). The induction of il-2 and ifng transcripts could be prevented by PJ34, but was independent of PARP1 and PARP2 (Fig. 6B and C), suggesting that the two PARP family members are redundant or that additional PJ34-sensitive ADP-ribosylating enzymes function in the transcriptional activation of T cells. The results indicate that PJ34 blocks T-cell activity by interfering with the autocrine, IL-2–driven positive feedback loop required for sustained T-cell proliferation, and suggest that ADP ribosylation is required for the initiation of the transcriptional program activating T-cell effector functions.

**Discussion**

Inhibitors of ADP-ribosylating enzymes have attracted attention lately as new compounds for cancer therapy (16, 17, 19, 20), and for the treatment of chronic inflammatory diseases and autoimmune pathologies (13–15, 32). We show here that a broad range inhibitor of ADP ribosylation, PJ34, is capable of preventing and also of curing the Helicobacter–induced, T cell–driven immunopathology that precedes gastric cancer development. Several pieces of evidence indicate that pathogenic T cells, and not Helicobacter-infected epithelial cells (which also upregulate PAR in the infectious process), are the key targets of PJ34 inhibition in our preclinical model: (a) The gastric expression of IFN-γ, which is produced...
exclusively by infiltrating CD4+ T cells, is strongly reduced in vivo; (b) the priming and polarization of T(H)1 T cells in the gut-draining lymph nodes is impaired both in vivo and ex vivo in cocultures of T cells with Helicobacter antigen–pulsed MLN-derived dendritic cells; (c) IFN-γ secretion by explanted MLN cells from infected mice cultured ex vivo is blocked, and (d) these effects are presumably due to the PJ34 sensitivity of il-2 gene transcription as shown in an ex vivo model of T-cell stimulation.

ADP-ribose polymers are detectable in T cells only hours after their activation by CD3/CD28 cross-linking; the time frame of PAR formation suggests a role for PAR in the transcriptional activation of genes encoding IL-2, IFN-γ, and the IL-2 receptor α-chain. In line with this observation, the inhibition of ADP ribosylation in T cells by PJ34 blocks il-2 and ifng gene transcription and, presumably as a consequence of these early effects, prevents T-cell proliferation in response to CD3/CD28 cross-linking– and PMA/ionomycin-induced activation. Our ex vivo results suggest that PARP1, PARP2, and possibly other PJ34-sensitive PARP family members have redundant roles during T-cell activation. Our finding that IL-10−/−/PARP1−/− mice develop gastritis and epithelial pathology with similar kinetics as the parental IL-10−/− PARP1+ strain argues in favor of a redundant role of PARP enzymes also in vivo. We conclude from the combined results that ADP ribosylation by PJ34-sensitive enzymes is an essential step in the activation and/or T(H)1 polarization of pathogenic T cells, which directly cause the gastric pathology preceding gastric neoplasia.

The concept of treating T cell–driven diseases such as chronic inflammatory disorders or autoimmune diseases with inhibitors of ADP-ribosylating enzymes is not new. PARP inhibitors have for instance been successfully used in preclinical models of asthma and colitis (13, 14, 33–35). PARP inhibition and PARP1 gene deletion were shown to protect mice from ovalbumin-induced allergic airway inflammation (14). In rodent models of Crohn’s disease–like colitis, the inhibition of ADP ribosylation was sufficient to prevent disease (13, 33–35). Protection was accompanied by decreased levels of inflammatory and T cell–derived cytokines.
and a restoration of colonic barrier functions (13, 33–35). Overall, substantial preclinical evidence is available to support a role for ADP ribosylation also in other T cell–driven immune and autoimmune pathologies, suggesting a more general mechanism and a broad applicability of ADP ribosylation inhibitors to these indications.

One of the least anticipated findings of our study was the almost complete reversion of preexisting Helicobacter–induced gastric lesions by PJ34 treatment. This result indicates that inhibitors of ADP ribosylation, for which safety data are now available due to their extensive testing in trials of metastatic breast and other cancers (19, 20), might be useful compounds for the treatment of symptomatic H. pylori–infected patients. Most patients presenting with atrophic gastritis, metaplasia, or dysplasia are routinely subjected to eradication therapy targeting the underlying infection; however, eradication is only partly efficient in reversing atrophy and fails in the treatment of metaplasia and dysplasia (29). As patients with any of these conditions have an at least 10-fold increased risk of developing gastric cancer compared with histologically unapparent infected individuals, and are currently subjected to an unsatisfactory watch-and-wait strategy, there is a clear unmet need for new treatment options for this patient group. We conclude based on the results obtained by our combined treatment with PJ34 and antibiotics that this inhibitor, or other compounds mimicking NAD+, may successfully reverse gastric preneoplastic lesions in humans if administered together with or after eradication therapy. In summary, we provide evidence that ADP ribosylation is required for T cell activation, and that inhibition of ADP-ribosylating enzymes efficiently impairs the TIR-1–polarized T-effector cell responses and the associated gastric immunopathology that are a hallmark of chronic Helicobacter infection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dirk Bumann, Christoph Dehio, Josef Jiricny, Isabelle Arnold, Iris Hitzler, and Ayca Sayi for helpful discussions.

Grant Support

Swiss National Science foundation grants 310030-127589 and 3100A0-113452 (A. Müller) and 31-109315.05 and 31-122421 (M. Altmeyer). Additional funding was supplied by the University Research Priority Program in Systems Biology/Functional Genomics, the UBS foundation, the Swiss Cancer League, and the Nils and Desiree Yde foundation (A. Müller) and by the Kanton of Zurich (M.O. Hottiger).

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Received 02/13/2010; revised 04/27/2010; accepted 05/03/2010.

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Isabella M. Toller, Matthias Altmeyer, Esther Kohler, et al.

Cancer Res 2010;70:5912-5922.

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