Aberrant Mucosal Mast Cell Protease Expression in the Enteric Epithelium of Nematode-Infected Mice Lacking the Integrin $\alpha_v\beta_6$, a Transforming Growth Factor- β_1 Activator

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Infection of mice with the nematode *Trichinella spiralis* triggers recruitment and differentiation of intraepithelial intestinal mucosal mast cells expressing mouse mast cell protease 1 (Mcpt-1), which contributes to expulsion of the parasite. Expression of Mcpt-1 is transforming growth factor (TGF)- β_1 -dependent *in vitro*. TGF- β_1 , which is secreted within tissues as a biologically inactive complex with latency-associated peptide, requires extracellular modification to become functionally active. The integrin- $\alpha_v\beta_6$ mediates local activation of TGF- β_1 in association with epithelia. Using *T. spiralis*-infected $\beta_6^{-/-}$ mice, we show accumulation of mucosal mast cells in the lamina propria of the small intestine with minimal recruitment into the epithelial compartment. This was accompanied by a coordinate reduction in expression of both Mcpt-1 and -2 in the jejunum and increased tryptase expression, whereas Mcpt-9 became completely undetectable. In contrast, the cytokine stem cell factor, a regulator of mast cell differentiation and survival, was significantly up-regulated in *T. spiralis*-infected $\beta_6^{-/-}$ mice compared with infected $\beta_6^{+/+}$ controls. Despite these changes, $\beta_6^{-/-}$ mice still appeared to expel the worms normally. We postulate that compromised TGF- β_1 activation within the gastrointestinal epithelial compartment is a major, but not the only, contributing factor to the observed changes in mucosal mast cell protease and epithelial cytokine expression in $\beta_6^{-/-}$ mice. (*Am J Pathol* 2007, 171:1237-1248; DOI: 10.2353/ajpath.2007.061245)

As a stereotypic TH2-regulated response to almost all gastrointestinal nematode infections of mammals, the population of mucosal mast cells (MMC) expands rapidly both in the mucosa and within the epithelium.¹⁻³ Intraepithelial MMCs are morphologically distinct from mast cells in nonmucosal sites and also differ in their content of granule mediators.^{2,3} The intraepithelial accumulation of a high proportion of MMCs is most obvious in the mouse but occurs in many other species as well, including parasitized rats, ruminants, and equids. Studies using mast cell-deficient mice have established the importance of MMC in resistance against some, but not all, nematode parasites.⁴⁻⁷ In mice, the highly soluble MMC granule-derived β -chymase mouse mast cell protease-1 (Mcpt-1) is abundantly expressed in the parasitized gut and is released both systemically and into the gut lumen during worm expulsion. BALB/c mice lacking Mcpt-1 (Mcpt-1^{-/-} BALB/c F₁₀) show significantly impaired expulsion of the intraepithelial nematode *Trichinella spiralis* in comparison to Mcpt-1^{+/+} BALB/c F₁₀ controls.⁸ Several studies suggest that a major role of MMC β -chymases is to promote epithelial permeability,^{9,10} and this may contribute to intestinal enteropathy.¹¹

The multifunctional cytokine transforming growth factor (TGF)- β_1 is a key regulator of Mcpt-1 expression and secretion *in vitro*,¹²⁻¹⁵ as well as having chemotactic properties for mast cells.¹⁶ TGF- β_1 is secreted by many cell types, and the nascent protein is usually biologically inactive because it is complexed with latency-associated peptide (LAP). TGF- β_1 -LAP binds latent TGF- β -binding protein 1 (LT-BP1) and may also bind to LT-BP3/LT-BP4 in the extracellular matrix, described as the large latent

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complex. In the intestine, TGF- β_1 is produced by enterocytes, promoting epithelial repair^{17,18} and reducing microbial^{19–21} and inflammatory bowel disease-associated enteropathy.^{21–24} Latent TGF- β_1 -LAP complex can be activated by a diverse range of factors. These include proteolysis by plasmin, metalloproteases, and cathepsins, and activation via TGF- β_1 -LAP-binding molecules including thrombospondin-1 and the α_v integrin family ($\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_6$, and $\alpha_v\beta_8$).^{25,26} Studies of null mice support roles for both integrin $\alpha_v\beta_6$ and TSP-1 as potential activators of TGF- β_1 *in vivo*^{27–29} and integrin $\alpha_v\beta_6$ is expressed almost exclusively by epithelial cells.^{29–31} We have confirmed the presence of β_6 integrin transcripts in enterocytes of the jejunum in normal and *T. spiralis*-infected S129 mice, where it is coexpressed with TGF- β_1 .³² Integrin $\alpha_v\beta_6$ binds LAP and, in the presence of both intracellular β -actin and LT-BP1, activates TGF- β_1 .^{25,33} Furthermore, integrin $\beta_6^{-/-}$ mice show exaggerated lung inflammation,^{28,34} which is reversed by epithelial expression of the human β_6 transgene.³⁵ Integrin $\beta_6^{-/-}$ /TSP-1 $^{-/-}$ double-null mice exhibited a significantly higher incidence of tissue inflammation in the lung, glandular stomach epithelium, and other tissues than wild-type or single-null mice.²⁹

In $\beta_6^{-/-}$ mice infected with the lumen-dwelling nematode *Nippostrongylus brasiliensis*, both MMC recruitment and Mcpt-1 expression were virtually ablated.³² In contrast, infection of $\beta_6^{-/-}$ mice with *T. spiralis*, a nematode that parasitizes jejunal epithelium, resulted in greatly enhanced MMC recruitment into the lamina propria with significantly reduced recruitment to the epithelial compartment and greatly reduced expression of the integrin $\alpha_E\beta_7$ by the aberrantly located MMC.³⁶ Here, we demonstrate that there is a coordinate reduction in expression of both Mcpt-1 and -2 by MMC in $\beta_6^{-/-}$ mice and that Mcpt-9 transcripts become completely undetectable. Interestingly, the cytokine stem cell factor (SCF), a key regulator of gastrointestinal nematode-induced MMC hyperplasia,^{37,38} is highly up-regulated in the epithelial compartment of $\beta_6^{-/-}$ mice compared with $\beta_6^{+/+}$ controls. We postulate that the aberrant responses to *T. spiralis* infection in the $\beta_6^{-/-}$ gastrointestinal mucosa are, in part, a result of compromised local TGF- β_1 activation within the epithelium rather than any systemic effects.

Materials and Methods

Transgenic Mice, Parasite Infections, and Sample Preparation

All experimental procedures involving laboratory animals were approved by The University of Edinburgh's Biological Services ethical review committee and were performed under license, as required by the United Kingdom's Animals (Scientific Procedures) Act 1986. Integrin- β_6 -null (S129 strain background: $\beta_6^{-/-}$) mice (Huang et al²⁸) were originally obtained from Dr. Kairbaan Hodivala-Dilke (Cell Adhesion and Disease Laboratory, GKT School of Medicine, St. Thomas' Hospital,

London, UK) and backcrossed with S129 $\beta_6^{+/+}$ controls (B&K Universal, Hull, UK) as previously described.³⁶ S129 $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice, 8- to 15-week-old and age- and sex-matched, were infected by gavage with 250 muscle larvae, and groups ($n = 4$ to 6) were sacrificed at various time points after infection for sample preparation and assessment of parasite burden. Maintenance of and infection with *Trichinella spiralis* larvae, sample collection for histology, total RNA and protein, recovery of muscle larvae and/or adult worms, were performed as described by us previously.^{8,32} For preparation of RNA from intestinal epithelium, mice were sacrificed on day 0 (uninfected) and day 12 after infection (worm expulsion phase) and epithelium purified and assessed for cellular composition as described previously.^{39,40}

Histology and Immunocytochemistry

Toluidine blue and esterase staining were performed as described previously.⁴¹ Immunohistochemical staining of paraffin-embedded paraformaldehyde-fixed sections for Mcpt1, Mcpt2, and tryptase were performed according to published protocols.^{36,42}

Mast Cell Cultures

Bone marrow-derived mast cells (BMMCs) from $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice were generated in the presence of recombinant mouse interleukin (IL)-3 (1 ng/ml; R&D Systems, Abingdon, UK), recombinant mouse IL-9 (5 ng/ml; R&D Systems Europe Ltd., Abingdon, UK), recombinant mouse SCF (50 ng/ml; PeproTech EC Ltd., London, UK), and recombinant human TGF- β_1 (1 ng/ml; Sigma-Aldrich, Poole, UK) as described previously.¹⁵ Viability was assessed using 0.2% nigrosin (Sigma-Aldrich) exclusion, and Mcpt-1 and -2 expression was assayed by enzyme-linked immunosorbent assay (ELISA) for up to 14 days *ex vivo*. Cytospins were prepared at 2- to 3-day intervals and stained with anti-Mcpt-1 antibody as described previously.⁴² To assay the effects of TGF- β_1 on BMMC protease transcription, BALB/c BMMCs were generated in the presence of recombinant mouse IL-3, IL-9, and SCF as described above with the addition of either recombinant human TGF- β_1 (1 ng/ml; Sigma-Aldrich) or 1 μ g/ml mouse anti-TGF- β_1 IgG₁ (clone 1D11; R&D Systems).¹⁵ Samples for RNA extraction were collected from both sets of experiments as described previously.³⁶

Frequencies of Mast Cell Progenitors (MCPs) in $\beta_6^{-/-}$ and $\beta_6^{+/+}$ Bone Marrow

The MCP frequencies were determined by limiting dilution following a modification of a published method.⁴³ Briefly, bone marrow cells from $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice were resuspended at 10^7 cells/ml in TI3S (1 ng/ml of recombinant human TGF- β_1 , 5 ng/ml of recombinant mouse IL-9, 1 ng/ml of recombinant mouse IL-3, and 50

Table 1. Primer Sequences Used in Standard RT-PCR for Identifying Protease Gene Transcripts

Gene	Primers	PCR product size
<i>Mcpt-1</i> *	Forward: 5'-GGAAACTGGAGAGAAAGAACCTAC-3' Reverse: 5'-GACAGCTGGGACAGAATGGGG-3'	460 bp
<i>Mcpt-2</i> *	Forward: 5'-ATTTCATGCGCTAGTTCCTCTGAC-3' Reverse: 5'-CAGGATGAGAACAGGCTGGGAT-3'	525 bp
<i>Mcpt-5</i> *	Forward: 5'-GGCAGAACAACGTGAATGAGCC-3' Reverse: 5'-AAGAACCTTCTGGAAGCTCAGGG-3'	418 bp
<i>Mcpt-6</i>	Forward: 5'-GCTCCTCTCTTTGAACCGGATC-3' Reverse: 5'-GGTGGGAGAGGCTCGTCATTA-3'	212 bp
<i>Mcpt-7</i>	Forward: 5'-GGCTGGGGTAACATCGACAAT-3' Reverse: 5'-CAAGTAATAGGTGACCCGGGTGTA-3'	317 bp
<i>Mcpt-9</i>	Forward: 5'-TTCCAAGTTCAATGACATCGTATTAC-3' Reverse: 5'-GATACTTTTTCCTCCAGTTCGC-3'	159 bp
<i>CPA</i> *	Forward: 5'-ACACAGGATCGAATGTGGAG-3' Reverse: 5'-TAATGCAGGACTTCATGAGC-3'	689 bp
SCF (KL-1 and KL-2) [†]	Forward: 5'-TCCGAAGAGGCCAGAACTA-3' Reverse: 5'-CAACTGCCCTTGTAAGACTTGA-3'	368 bp (KL-1) 284 bp (KL-2)
α_V	Forward: 5'-GAAGTTACTTCGGATTCCGCCG-3' Reverse: 5'-CATCTTTGGCATAATCTCGATTGC-3'	203 bp
β_6 [‡]	Forward: 5'-CAGTTCTGACATTGTTTCAGA-3' Reverse: 5'-AGTTAATGGCAAAATGTGCT-3'	356 bp
ATP5a (ATP synthase H: <i>ATP5a1</i>)	Forward: 5'-CTGTACTGCATCTACGTCGCGA-3' Reverse: 5'-AGCCTGCTTGGATAGATCGTCATA-3'	230 bp

*Primer sequences from Wastling et al.⁴¹

[†]Primers designed to distinguish between the two splice variants of SCF.

[‡]Primer sequences from Huang et al.²⁸

ng/ml of recombinant mouse SCF). Serial dilutions, equivalent to 10^5 , 10^4 , 10^3 , and 10^2 cells/well, were prepared in T13S and plated out in replicates of 24 in 96-well plates. After 7 days wells were fed with 50 μ l of T13S and incubated for a further 7 days, after which cultures were harvested, freeze-thawed, and assayed for the presence of mature MMCs by Mcpt-2 ELISA.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Analysis

Extraction of total RNA from jejunum, BMMCs, or isolated epithelium, and purification and removal of contaminating DNA using DNA-free DNase (Ambion Inc., Austin, TX) has been described previously.^{32,36} For RT-PCR analysis, 1 μ g of total RNA was reverse-transcribed as previously described, and 1/20th volume was amplified by PCR using gene-specific primers, with equivalent quantities of nonreverse-transcribed RNA as negative controls.⁸ All reaction conditions were optimized to ensure the number of thermocycles used correlated with the amplification stage of the PCR. Primer sequences and product sizes are given in Table 1. Unless previously published, primers were designed from sequences in GenBank using the Primer 3 program available on <http://frodo.wi.mit.edu/cgi-bin/primer3/primer3 WWW.cgi>, checking specificity by searching the NCBI GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov>). Amplifications for Mcpt-1, Mcpt-2, Mcpt-5, Mcpt-6, Mcpt-7, and carboxypeptidase A were performed for 40 seconds at 94°C, 40 seconds at 63°C, and 120 seconds at 72°C for 26 (Mcpt1, -2, -5), 30 (Mcpt-6, carboxypeptidase A), or 32 thermocycles

(Mcpt-7) as appropriate. Amplifications for ATP5a, Mcpt-9, and SCF (KL-1/KL-2) were performed for 40 seconds at 94°C, 40 seconds at 55°C, and 120 seconds at 72°C for 28 thermocycles (ATP5a), 34 thermocycles (Mcpt-9), or 40 thermocycles (SCF KL-1/KL-2). Amplifications for α_V and β_6 integrin subunits were performed for 40 seconds at 94°C, 40 seconds at 52°C, and 120 seconds at 72°C for 32 thermocycles (α_V) or 36 thermocycles (β_6). PCR products were visualized on ethidium bromide-stained 1.6% agarose gels and images recorded and analyzed using a Kodak Digital Science Image Station 440CF and 1D Image Analysis software (Eastman-Kodak, Rochester, NY). To confirm the identity of the PCR products, some gels were subjected to Southern blotting following standard protocols and hybridization using digoxigenin-labeled internal gene-specific probes as described previously.⁴¹

Real-Time RT-PCR Analysis of Transcripts in Epithelial RNA

Standard housekeeping genes (*GAPDH*, creatine kinase, β -actin, *ATP5a*) were found to vary significantly in isolated epithelium (P.A.K., unpublished observations),⁴⁰ precluding the use of the formula described above for analyzing transcripts in isolated epithelium. We therefore set up quantitative real-time RT-PCR assays for the cytokines SCF, IL-4, IL-7, IL-13, and TGF- β_1 , generating PCR products to act as standards. All primer sequences and product sizes are given in Table 2. PCR products to act as standards for each transcript were generated from reverse-transcribed $\beta_6^{+/-}$ -isolated epithelium (day 12 after infection), using the ex-

Table 2. Primer Sequences Used in Real-Time PCR for Identifying Cytokine Transcripts

Gene	Internal primers	PCR product size	External primers	PCR product size
<i>SCF</i>	Forward: 5'-GAAGTCAGTCTTTTCCTTGAC AGT-3' [*] Reverse: 5'-GCATGTCACATTATACTATTGCAA ACA-3' [*]	70 bp [†]	Forward: 5'-ACGTGGACCAGTGAAGAAC-3' Reverse: 5'-TTGCACATTTCAGCATTCCTC-3'	181 bp [†]
<i>IL-4</i>	Forward: 5'-ACAGGAGAAGGGACGCCAT-3' [‡] Reverse: 5'-GAAGCCCTACAGACGAGCTCA-3' [‡]	95 bp	Forward: 5'-CCCCAGCTAGTTGTCATCCT-3' Reverse: 5'-GCATGGAGTTTCCCATGTT-3'	250 bp
<i>IL-13</i>	Forward: 5'-AGACCAGACTCCCCTGTGCA-3' [‡] Reverse: 5'-TGGGTCCTGTAGATGGCATTG-3' [‡]	123 bp	Forward: 5'-TTGAGGAGCTGAGCAACATC-3' Reverse: 5'-GGTTACAGAGGCATGCAAT-3'	173 bp
<i>IL-7</i>	Forward: 5'-ATTATGGGTGGTGAGAGCCG-3' [‡] Reverse: 5'-GTTCCTGTCATTGTGTCCA ATTCA-3' [‡]	231 bp	Forward: 5'-GGAGTCAGCTGCCTGAACTG-3' Reverse: 5'-TGGTTCATTATTCGGGCAAT-3'	334 bp
<i>TGF-β₁</i>	Forward: 5'-TGACGTCAGTGGAGTTGTACGG-3' [‡] Reverse: 5'-GGTTCATGTCATGGATGGTGC-3' [‡]	140 bp	Forward: 5'-ATACGCCTGAGTGGCTGTCT-3' Reverse: 5'-GCCATGAGGAGCAGGAAG-3'	214 bp

For production of PCR products as standards, external primers were used to generate RT-PCR products slightly larger than that generated by the internal primers used in the assay.

^{*}Primer sequences from Sun et al.⁶⁹

[†]Identical from both splice variants.

[‡]Primer sequences from Overbergh et al.⁷⁰

ternal primer sets, carrying out amplifications for 40 seconds at 94°C, 40 seconds at 55°C, and 120 seconds at 72°C for 40 thermocycles. PCR products were purified using a Roche High Pure PCR product purification kit (Roche, Lewes, UK) according to the manufacturer's instructions. PCR products were quantified and 10-fold dilutions set up to generate a standard curve, checking efficiency in the real-time PCR assays with the internal primers.

For real-time RT-PCR analysis, 1 μg of total RNA from isolated epithelium from $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice collected on day 0 and day 12 after infection was reverse-transcribed as previously described.⁸ One hundred ng of cDNA or appropriate concentration of standard was amplified using 0.3 μmol/L of primers in 20 μl of SYBR Green master mix (2.5 mmol/L MgCl₂) (Qiagen, Valencia, CA). PCR conditions were as follows: 95°C for 15 minutes, then 50 thermocycles of 94°C for 15 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. Reactions were performed in triplicate. Melting curves were calculated for each reaction to confirm identity of the PCR product and to ensure primer-dimer formation did not occur. Master mix only controls were included in all reactions as blanks, along with RNA only controls to check for DNA contamination. Cycle thresholds (Ct) and copy number were calculated using Opticon 2 software, correcting fluorescence values against background and blanks, with

subsequent analysis performed in Microsoft Excel and using the InStat statistics package (GraphPad Software, Inc., San Diego, CA).

Results

MMC Distribution and Granule Protease Expression in Normal and Parasitized Jejunum of $\beta_6^{-/-}$ and $\beta_6^{+/+}$ Mice

Age- and sex-matched $\beta_6^{-/-}$ and $\beta_6^{+/+}$ S129 mice ($n = 4$ to 6 mice/group/time point) were each infected with 250 *T. spiralis* L3 in four separate experiments to i) investigate infection kinetics and Mcpt-1 quantification by ELISA (experiments 1 and 2), ii) provide material for histological examination of tissues (experiment 3), and iii) isolate jejunal epithelium (experiment 4, day 0 and day 12 after infection only).

Recruitment of esterase⁺ MMCs to the jejunal epithelium increased significantly during infection in S129 $\beta_6^{+/+}$ mice, whereas recruitment to this site was significantly compromised in infected $\beta_6^{-/-}$ mice (Table 3 and Figure 2a). In $\beta_6^{-/-}$ mice, MMCs accumulated in the lamina propria during infection as described previously.³⁶ In the current study, the numbers of toluidine blue⁺ and esterase⁺ MMCs were very similar (data not shown). Reduced recruitment of MMCs into the epithe-

Table 3. Esterase⁺ MMC per Villus Crypt Unit (vcu) (±SE) in the Jejunum from $\beta_6^{+/+}$ and $\beta_6^{-/-}$ Mice on Days 0 (Uninfected), 12, 16, and 28 after Infection with *T. spiralis* ($n = 4$ to 6)

	Epithelium		Lamina propria	
	$\beta_6^{+/+}$	$\beta_6^{-/-}$	$\beta_6^{+/+}$	$\beta_6^{-/-}$
Day 0	0.11 (±0.11)	0.05 (±0.06)	0.04 (±0.06)	0.08 (±0.06)
Day 12	9.44 (±3.2)	1.45 (±0.30) [†]	1.92 (±0.91)	3.56 (±3.1) [†]
Day 16	8.05 (±1.03)	1.22 (±0.29) [†]	2.46 (±0.65)	8.7 (±1.61) [*]
Day 28	5.22 (±0.51)	1.7 (±0.47) [†]	2.45 (±0.77)	8.29 (±1.36) [†]

^{*} $P < 0.05$, [†] $P < 0.01$; $\beta_6^{+/+}$ versus $\beta_6^{-/-}$ Mann-Whitney nonparametric test.

Figure 1. Sections of jejunum from *T. spiralis*-infected (day 12 after infection) $\beta_6^{+/+}$ (A, C, and E) and $\beta_6^{-/-}$ (B, D, and F) mice stained for Mcpt-1 (A and B), Mcpt-2 (C and D), and tryptase (E and F). Staining techniques are described in Materials and Methods. Positively stained MMCs are indicated by **arrows**. Note the decreased numbers of intraepithelial Mcpt-1⁺ and Mcpt-2⁺ MMCs, and increased numbers of tryptase⁺ MMCs, in $\beta_6^{-/-}$ mice compared with controls. Scale bars = 25 μ m.

lium of $\beta_6^{-/-}$ mice was associated with significantly ($P \leq 0.01$) reduced numbers of Mcpt-1⁺ and Mcpt-2⁺ MMCs and with enhanced numbers of tryptase⁺ cells in the lamina propria ($P \leq 0.05$) (Figures 1 and 2, b–d). This was concomitant with significantly ($P \leq 0.01$) reduced expression of Mcpt-1 and Mcpt-2 in jejunal ex-

tracts (Figure 2, e and f). Transcripts for Mcpt-1 and Mcpt-2 were reduced, and Mcpt-5 and Mcpt-6 were enhanced in $\beta_6^{-/-}$ jejunum, whereas there were no obvious differences in Mcpt-7 and carboxypeptidase A (Figure 3a). Interestingly, transcripts for Mcpt-9, which, like those for Mcpt-1 and Mcpt-2, are up-regulated during nem-

Figure 2. Mast cell counts in the jejunum from uninfected (day 0) and *T. spiralis*-infected (day 12 after infection) $\beta_6^{+/+}$ (filled bars) and $\beta_6^{-/-}$ (open bars) mice (+SE) stained for esterase (a), tryptase (b), Mcpt-1 (c), and Mcpt-2 (d), and levels of Mcpt-1 (e) and Mcpt-2 (f) in the jejunum measured by ELISA, as described in Materials and Methods. * $P < 0.05$, ** $P < 0.01$; Mann-Whitney nonparametric test. Note that reduced recruitment of MMCs into the epithelium of $\beta_6^{-/-}$ mice is associated with significantly reduced Mcpt-1 and Mcpt-2 expression and enhanced tryptase expression in the lamina propria.

atode infection by intraepithelial MMCs,^{44,45} were completely undetectable in $\beta_6^{-/-}$ jejunum (Figure 3a) and in jejunal epithelium (Figure 3b), whereas Mcpt-9 was up-regulated in $\beta_6^{+/+}$ jejunal epithelium during *T. spiralis* infection, concomitantly with Mcpt-1 (Figure 3, a and b).

In Vitro Expression of Granule Proteases by BMMCs—the Role of TGF- β_1

Previous studies have established a major role for TGF- β_1 in the differentiation of bone marrow cells into MMC homologues expressing Mcpt-1 and -2.^{12–15} To determine whether the aberrant protease phenotype of the MMCs recruited into the lamina propria might be attributed entirely to lack of active TGF- β_1 , or whether there are additional factors operating within the mucosa, we compared protease profiles in BMMCs cultured for 14 days in IL-3, IL-9, and SCF in the presence (TGF- β^+) or absence (TGF- β^-) of exogenous TGF- β_1 and with the addition of anti-TGF- β_1 antibody to block endogenous TGF- β_1 . Although transcripts for Mcpt-1 and Mcpt-2 were almost undetectable in TGF- β^- cultures, there were no obvious differences in levels of transcription of Mcpt-5, -6, -7, and -9 (Figure 3c). Real-time RT-PCR confirmed that transcription of Mcpt-9 was independent of TGF- β_1 *in vitro* (data not shown), and therefore its down-regulation in the $\beta_6^{-/-}$ jejunum seems to be unrelated to activation of TGF- β_1 .

Figure 3. a: RT-PCR products for Mcpt-1 (M1), Mcpt-2 (M2), Mcpt-5 (M5), Mcpt-6 (M6), Mcpt-7 (M7), Mcpt-9 (M9), carboxypeptidase A (CPA), and housekeeping gene (ATP5a) (HG) from RNA isolated from $\beta_6^{-/-}$ and $\beta_6^{+/+}$ jejunum on day 12 of infection. Each lane represents a sample from a single mouse ($n = 4$ per group). Note transcripts for Mcpt-1 (M1) and Mcpt-2 (M2) are reduced, and Mcpt-5 (M5) and Mcpt-6 (M6) are enhanced in $\beta_6^{-/-}$ jejunum, whereas there were no obvious differences in Mcpt-7 (M7) and carboxypeptidase A (CPA). Interestingly, transcripts for Mcpt-9 (M9) were completely undetectable in $\beta_6^{-/-}$ jejunum. b: RT-PCR products for Mcpt-9 (M9) and housekeeping gene (ATP5a) (HG) from RNA isolated from epithelium from $\beta_6^{-/-}$ and $\beta_6^{+/+}$ jejunum of uninfected controls (day 0) and from day 12 of infection. Each lane represents a sample from a single mouse ($n = 4$ per group). Note that transcripts for Mcpt-9 (M9) were up-regulated in the jejunal epithelium of $\beta_6^{+/+}$ mice but were completely undetectable in $\beta_6^{-/-}$ epithelium. c: RT-PCR products for Mcpt-1 (M1), Mcpt-2 (M2), Mcpt-5 (M5), Mcpt-6 (M6), Mcpt-7 (M7), Mcpt-9 (M9), and housekeeping gene (ATP5a) (HG) from RNA isolated from BMMCs cultured for 14 days in IL-3, IL-9, SCF, and TGF- β_1 or in the absence of TGF- β_1 , and the addition of anti-TGF- β antibody ($n = 4$ per group; see Materials and Methods for details). Note that transcripts for Mcpt-1 (M1) and Mcpt-2 (M2) are almost undetectable in the absence of TGF- β_1 , whereas there was no obvious difference in levels of transcripts for Mcpt-5 (M5), Mcpt-6 (M6), Mcpt-7 (M7), and Mcpt-9 (M9).

Effect of the β_6 Integrin Deletion on Mast Cells in Stomach, Colon, and Ear Pinnae

To determine whether the absence of β_6 integrin affected mast cell distribution throughout the gastrointestinal tract,

Table 4. Numbers of Mast Cells in the Stomach, Colon, and Ear Pinna from Uninfected (Day 0) and *T. spiralis*-Infected (Day 12 after Infection) $\beta_6^{+/+}$ and $\beta_6^{-/-}$ Mice ($\pm$ SE)

	Day 0		Day 12	
	$\beta_6^{+/+}$	$\beta_6^{-/-}$	$\beta_6^{+/+}$	$\beta_6^{-/-}$
Stomach				
Esterase ⁺	5.63 ($\pm$ 0.97)	38.5 ($\pm$ 7.8)*	51.6 ($\pm$ 7.7)	65.7 ($\pm$ 7.3)
Mcp-1 ⁺	0.1 ($\pm$ 0.1)	0.0 ($\pm$ 0.0)	25.7 ($\pm$ 5.9)	2.7 ($\pm$ 0.7) [†]
Mcpt-2 ⁺	3.9 ($\pm$ 1.2)	3.8 ($\pm$ 0.6)	37.5 ($\pm$ 10.3)	22.5 ($\pm$ 5.4)
Tryptase ⁺	29.4 ($\pm$ 5.5)	31.7 ($\pm$ 6.9)	25.3 ($\pm$ 6.3)	57.9 ($\pm$ 13.0)
Colon				
Esterase ⁺	2.3 ($\pm$ 0.9)	2.7 ($\pm$ 0.4)	65.8 ($\pm$ 27.2)	19.3 ($\pm$ 2.2)*
Mcp-1 ⁺	0.42 ($\pm$ 0.2)	0 ($\pm$ 0)	59.6 ($\pm$ 25.5)	0.67 ($\pm$ 0.3)*
Mcpt-2 ⁺	0.2 ($\pm$ 0.2)	0.3 ($\pm$ 0.2)	20.8 ($\pm$ 19.6)	6.2 ($\pm$ 4.0)
Tryptase ⁺	29.4 ($\pm$ 5.5)	31.8 ($\pm$ 6.9)	25.3 ($\pm$ 6.3)	57.9 ($\pm$ 13)
Ear pinna				
Esterase ⁺	104.8 ($\pm$ 14.9)	98.67 ($\pm$ 7.1)	84.8 ($\pm$ 6.7)	91.25 ($\pm$ 8.9)
Mcp-1 ⁺	0.0 ($\pm$ 0.0)	0.0 ($\pm$ 0.0)	0.0 ($\pm$ 0.0)	0.0 ($\pm$ 0.0)
Mcpt-2 ⁺	0.0 ($\pm$ 0.0)	0.0 ($\pm$ 0.0)	0.0 ($\pm$ 0.0)	0.0 ($\pm$ 0.0)
Tryptase ⁺	29.4 ($\pm$ 5.5)	47.7 ($\pm$ 8.6)	41.3 ($\pm$ 2.8)	39.2 ($\pm$ 9.3)

$\beta_6^{+/+}$ versus $\beta_6^{-/-}$; * $P < 0.05$, [†] $P < 0.01$; Mann-Whitney nonparametric test.

the distribution of MMCs and the expression of their granule proteases were compared in the glandular stomach and in the colon of normal and $\beta_6^{-/-}$ mice. Uninfected $\beta_6^{-/-}$ mice have significantly ($P \leq 0.05$) higher baseline numbers of esterase⁺ MMCs in the glandular stomach than $\beta_6^{+/+}$ controls (Table 4), but there was no difference in the number of gastric MMCs in *T. spiralis*-infected $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice (day 12 after infection) (Table 4). This is in accordance with our previous observations with *N. brasiliensis*-infected $\beta_6^{-/-}$ mice.³² However, infected $\beta_6^{-/-}$ mice had significantly ($P \leq 0.05$) reduced numbers of Mcpt-1⁺ MMCs, with a trend toward fewer Mcpt-2⁺ and more tryptase⁺ MMCs (Table 4). $\beta_6^{+/+}$ mice showed a significant ($P \leq 0.05$) increase in numbers of esterase⁺, predominantly Mcpt-1⁺ MMCs in the colon after *T. spiralis* infection (Table 4). The population of MMCs in the colons of infected $\beta_6^{-/-}$ mice was significantly reduced compare to that of infected $\beta_6^{+/+}$ controls, and this was associated with reduced Mcpt-1 expression. To determine whether mast cells elsewhere in the body were affected, the numbers of esterase⁺ connective tissue mast cells in the ear pinnae were compared in *T. spiralis*-infected and uninfected $\beta_6^{-/-}$ and $\beta_6^{+/+}$ mice. The absence of β_6 had no effect on this population of connective tissue mast cells in the ear (Table 4).

Comparison of Cultured MMC Homologues Derived from Bone Marrow of $\beta_6^{-/-}$ and $\beta_6^{+/+}$ Mice

The possibility that systemic mechanisms contribute to the aberrant location and protease expression of MMCs in $\beta_6^{-/-}$ mice was investigated. One possibility is that MMCs in $\beta_6^{-/-}$ mice are inherently unable to differentiate and/or there is a difference in mast cell precursor (MCP) frequencies. To test this, we compared the development of MMC homologues from bone marrow cultures derived from $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice. The frequencies of bone marrow-derived MCPs were assessed using a modifica-

tion of a published technique,^{43,46} and bone marrow from $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice had similar MCP frequencies (Figure 4a). BMMCs from both strains were equivalent in their viability (Figure 4b) and their capacity to secrete Mcpt-1 and -2 into the culture supernatant (Figure 4c). There was equivalent expression of Mcpt-1 and Mcpt-2 in cell pel-

Figure 4. a: Limiting dilution analysis of MCP frequency in bone marrow from $\beta_6^{+/+}$ (filled bars) and $\beta_6^{-/-}$ (open bars) mice cultured in the cytokines described above, as assessed by Mcpt-2 ELISA (see Materials and Methods for details). NS, not significant. b: Viability of BMMCs from $\beta_6^{+/+}$ (filled bars) and $\beta_6^{-/-}$ (open bars) mice (+SE) cultured for 14 days in the presence of TGF- β_1 , IL-3, IL-9, and SCF (see Materials and Methods for details) measured using an Improved Neubauer counting chamber and nigrosin exclusion. c: ELISA analysis of Mcpt-2 secretion into BMMC culture supernatant. d: RT-PCR products for Mcpt-1 (M1), Mcpt-2 (M2), Mcpt-5 (M5), Mcpt-6 (M6), Mcpt-7 (M7), Mcpt-9 (M9), and housekeeping gene ATP5a (HG) from $\beta_6^{+/+}$ and $\beta_6^{-/-}$ BMMC RNA as indicated. e: RT-PCR products for α_V , β_6 , and ATP5a (HG) from $\beta_6^{+/+}$ and $\beta_6^{-/-}$ BMMC RNA as indicated, together with negative (no RT) and positive ($\beta_6^{+/+}$ epithelium) controls.

Figure 5. **a:** Abundance of SCF transcripts from RNA from $\beta_6^{+/+}$ (filled bars) and $\beta_6^{-/-}$ (open bars) isolated jejunal epithelium taken on day 0 and day 12 after *T. spiralis* infection, as quantified by real-time PCR (infected versus uninfected, $\beta_6^{+/+}$ versus $\beta_6^{-/-}$; * $P < 0.05$, Mann-Whitney nonparametric test. NS, not significant). **b:** RT-PCR products for KL-1 and KL-2 splice variants of SCF from RNA isolated from $\beta_6^{-/-}$ and $\beta_6^{+/+}$ epithelium on day 12 of *T. spiralis* infection, as indicated. Each lane represents a sample from a single mouse ($n = 4$ per group). **c:** Ratios of KL-1 RT-PCR products with respect to KL-2. See Materials and Methods for details.

lets harvested at day 14 (data not shown). BMMCs from $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice cultured in the presence of TGF- β_1 showed equivalent expression of the proteases Mcpt-1, -2, -5, -6, and -7 (Figure 4d), as well as the integrin α_v (Figure 4e), in accordance with previous data showing that integrin α_v is expressed by murine BMMCs.⁴⁷ $\beta_6^{+/+}$, but not $\beta_6^{-/-}$, BMMCs showed barely detectable expression of the integrin β_6 . Overall these results suggest that there is no deficiency at the level of the bone marrow that would explain the aberrant protease expression and distribution of MMCs in the parasitized jejunum of $\beta_6^{-/-}$ mice.

Expression of Cytokines in Normal and Parasitized $\beta_6^{-/-}$ and $\beta_6^{+/+}$ Jejunal Epithelium and Mucosa

SCF is vital for the generation of intestinal mastocytosis observed during *T. spiralis* infection^{37,38} and may be chemotactic for intestinal MMCs.⁴⁸ Because MMCs in wild-type mice predominantly localize in the epithelium and have access to SCF on the lateral membranes of enterocytes,⁴⁹ we examined the expression of this cytokine within the jejunal epithelium. Real-time PCR was used to quantify the abundance of transcripts for SCF in jejunal epithelium isolated from $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice on day 0 and on day 12 after infection with *T. spiralis* (Figure 5a). Transcripts encoding SCF were significantly up-regulated in the epithelium of *T. spiralis*-infected $\beta_6^{-/-}$ mice in comparison to the constitutive expression seen in $\beta_6^{+/+}$ controls ($P \leq 0.05$) (Figure 5a). There were no significant differences in the levels of SCF transcripts detected in whole jejunum from *T. spiralis*-infected $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice using real-time PCR (data not shown). RT-PCR using primers that distinguish between the two splice variants of SCF, KL-1 and KL-2, demonstrated an increased transcription of KL-1 with respect to KL-2 in $\beta_6^{-/-}$ epithelium (Figure 5, b and c).

Figure 6. Abundance of transcripts for IL-4 (a), IL-13 (b), IL-7 (c), and TGF- β_1 (d) as indicated, from RNA from $\beta_6^{+/+}$ (filled bars) and $\beta_6^{-/-}$ (open bars) isolated jejunal epithelium taken on day 0 and day 12 after *T. spiralis* infection, as quantified by real-time PCR (infected versus uninfected, $\beta_6^{+/+}$ versus $\beta_6^{-/-}$; * $P < 0.05$, Mann-Whitney nonparametric test. NS, not significant).

T. spiralis-induced MMC hyperplasia is dependent, among other factors, on the Th2 cytokines IL-4 and IL-13,^{50–52} which are also products of mast cells.^{53,54} Therefore we quantified the epithelial expression of these cytokines in comparison with TGF- β_1 and of IL-7, which is highly expressed by intestinal epithelial cells.⁵⁵ Transcripts for IL-4 and IL-13 were undetectable in uninfected $\beta_6^{+/+}$ and $\beta_6^{-/-}$ epithelium but increased significantly ($P \leq 0.05$) in both groups on day 12 of infection (Figure 6, a and b). Transcription of IL-4 and IL-13 was, however, comparable in the epithelium of $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice. Levels of TGF- β_1 remained constant in uninfected and *T. spiralis*-infected $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice (Figure 6d), in accordance with our previous observations.³² Interestingly, the epithelial cytokine IL-7 was significantly up-regulated in infected $\beta_6^{-/-}$ mice but remained at uninfected levels in $\beta_6^{+/+}$ controls (Figure 6c). This may relate to the fact that IL-7 mRNA can be down-regulated by TGF- β_1 in some cell types.⁵⁶

Comparison of TH2-Driven Inflammation and of CD3⁺ T-Cell Populations in the Parasitized Jejunum of $\beta_6^{-/-}$ and $\beta_6^{+/+}$ Mice

There is evidence to suggest that many components of a complex TH2-driven response, including goblet cell-derived mucins, locally derived T cells, and eosinophils play a role in elimination of worms in addition to MMCs and Mcpt-1.^{3,57–59} Therefore we assessed the numbers of goblet cells, eosinophils, and CD3⁺ T cells in the jejunum of *T. spiralis*-infected (day 12 after infection) and control $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice (Table 5). Goblet cell and eosinophil numbers increased to similar levels in infected $\beta_6^{-/-}$ jejunum and $\beta_6^{+/+}$ controls. This is in accordance with previous observations with *N. brasiliensis*-infected $\beta_6^{-/-}$ mice.³² There was no overall increase in CD3⁺ T cells in either group, also in accordance with previous observations.³² However, $\beta_6^{-/-}$ mice had reduced numbers of

Table 5. Numbers of Goblet Cells, Eosinophils, and CD3⁺ Lymphocytes in the Jejunum from Uninfected (Day 0) and *T. spiralis*-Infected (Day 12 after Infection) $\beta_6^{+/+}$ and $\beta_6^{-/-}$ Mice ($\pm$ SE)

	Day 0		Day 12	
	$\beta_6^{+/+}$	$\beta_6^{-/-}$	$\beta_6^{+/+}$	$\beta_6^{-/-}$
Goblet cells	9.84 ($\pm$ 0.34)	10.32 ($\pm$ 0.86)	16.98 ($\pm$ 1.77)	17.93 ($\pm$ 0.58)
Eosinophils	34.8 ($\pm$ 7.2)	57.9 ($\pm$ 15.5)	152.32 ($\pm$ 31.0)	159 ($\pm$ 32.1)
CD3 ⁺ lymphocytes				
Epithelium	8.74 ($\pm$ 0.9)	6.3 ($\pm$ 0.98)	11.05 ($\pm$ 2.12)	3.6 ($\pm$ 0.25) [†]
Lamina propria	4.05 ($\pm$ 0.38)	4.71 ($\pm$ 0.59)	5.77 ($\pm$ 0.65)	8.64 ($\pm$ 0.65) [*]

$\beta_6^{+/+}$ versus $\beta_6^{-/-}$; NS, not significant. Note that goblet cell and eosinophil numbers increase normally in the $\beta_6^{-/-}$ jejunum on infection, but $\beta_6^{-/-}$ mice had reduced numbers of CD3⁺ T cells in the epithelium with slightly increased numbers in the lamina propria.

^{*} $P < 0.05$, [†] $P < 0.01$; Mann-Whitney nonparametric test.

CD3⁺ T cells in the epithelium with slightly increased numbers in the lamina propria ($\beta_6^{+/+}$ versus $\beta_6^{-/-}$; $*P \leq 0.01$ day 12 after infection Mann-Whitney nonparametric test).

Expulsion Kinetics of *T. spiralis* from $\beta_6^{-/-}$ and $\beta_6^{+/+}$ Mice

There were no significant differences in mean worm burdens at any stage of infection, between $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice (Figure 7a), nor of total muscle larvae ($\beta_6^{+/+}$ 12,241 ($\pm$ 2181) $\beta_6^{-/-}$ 16,628 $\pm$ 4602; day 35 after infection (NS), despite the significantly reduced concentrations of Mcpt-1 in the serum of $\beta_6^{-/-}$ mice (Figure 7b) (days 13,

17, and 27; $\beta_6^{+/+}$ versus $\beta_6^{-/-}$; $*P \leq 0.05$; Mann-Whitney nonparametric test). To check the expulsion kinetics of the S129 background and to verify the time points chosen were appropriate, SPF S129 control mice were simultaneously infected with the same dosage (Figure 7a). Although worm burdens were significantly reduced by day 13 in comparison to day 6 ($P \leq 0.05$), in accordance with previous data from both S129 and BALB/c mice,⁶⁰ worms persisted until after day 20 after infection, indicating that *T. spiralis* worms are expelled more slowly in S129 mice than is generally observed in BALB/c mice at this dosage.^{8,60}

Discussion

Here, we show compromised transcription and expression of the MMC-specific proteases Mcpt-1 and Mcpt-2 in the jejunum of *T. spiralis*-infected $\beta_6^{-/-}$ mice. In contrast, expression of the MMC proteases Mcpt-5, Mcpt-6, and Mcpt-7, as measured by RT-PCR or anti-tryptase antibody, are unaffected or enhanced (Figures 1, 2, and 3a). These observations tally with the reduced recruitment of MMC to the epithelial compartment and their accumulation in the lamina propria, which increased during the course of infection (Table 3).³⁶ They are also consistent with the hypothesis that intraepithelial MMCs express the highly TGF- β_1 -dependent granule proteases Mcpt-1 and Mcpt-2^{12,13} because of their proximity to activated TGF- β_1 in association with integrin $\alpha_v\beta_6$ on the lateral membranes of enterocytes. The reduced expression of Mcpt-1 in the glandular stomach and colon (Table 4) suggests that integrin $\alpha_v\beta_6$ activation of TGF- β_1 may be important for epithelial MMC differentiation throughout the gastrointestinal tract, consistent with the epithelial expression of integrin $\alpha_v\beta_6$ in these tissues,^{29,30} which we have confirmed by RT-PCR (P.A.K., unpublished observations). However, as will be discussed below, there are a number of observations that indicate that the events in the epithelium are more complex than might be expected if the only function of $\alpha_v\beta_6$ on enterocytes was to activate latent TGF- β_1 .

With regard to the MMC population in the parasitized jejunum there were three striking findings associated with the absence of β_6 integrin: reduced expression of Mcpt-1 and -2, absence of expression of Mcpt-9, and failure of MMCs to enter the epithelium. We also see higher base-

Figure 7. a: Mean total worm burdens ($\pm$ SE) from S129 $\beta_6^{+/+}$ (solid line) and $\beta_6^{-/-}$ (dashed line) mice infected with 300 *T. spiralis* L3, typical of two experiments. Mean total worm burdens ($\pm$ SE) are also shown from S129 mice infected with 300 *T. spiralis* L3 at the same time, to show expulsion kinetics (dotted line) (infected versus uninfected; $*P < 0.05$, $**P < 0.01$; Mann-Whitney nonparametric test). **b:** Concentrations of Mcpt-1 in the serum (μ g/ml) from S129 $\beta_6^{+/+}$ (solid line) and $\beta_6^{-/-}$ (dashed line) mice infected with *T. spiralis* (as shown in Figure 4a) ($\beta_6^{+/+}$ versus $\beta_6^{-/-}$; $*P < 0.05$; Mann-Whitney nonparametric test).

line numbers of gastric MMCs in $\beta_6^{-/-}$ mice than in $\beta_6^{+/+}$ controls, which may reflect altered MCP development or recruitment. We therefore considered the possibility that the targeting of β_6 integrin might have unintended consequences for mast cell development, perhaps through effects of the mutation on an adjacent locus, which directly affected mast cell differentiation and gene expression. However, this was not supported by our *in vitro* observations on the growth and differentiation of MMC homologs. BMMCs from $\beta_6^{-/-}$ mice cultured in the presence of TGF- β_1 differentiate and express Mcpt-1 and Mcpt-2 and the integrin α_V at comparable levels with BMMCs from $\beta_6^{+/+}$ controls. It is worth noting that MCP frequency in bone marrow is similar in both $\beta_6^{-/-}$ and $\beta_6^{+/+}$ mice, and connective tissue mast cells in the ear pinna are unaffected by the deletion of β_6 integrin (Table 4 and Figure 4). Furthermore, Mcpt-9 was equally expressed by both $\beta_6^{+/+}$ and $\beta_6^{-/-}$ BMMCs cultured in the presence of TGF- β_1 . Together, these *in vivo* and *in vitro* observations strongly suggest that it is unlikely that targeting the β_6 integrin has any direct effect on MMCs or their precursors.

It is unlikely that the failure to activate TGF- β_1 is the sole mechanism that drives the aberrant mast cell response. Mcpt-9 is reported to be regulated *in vitro* by IL-10 and SCF,⁴⁴ but in our studies transcript levels for Mcpt-9 were similar in cultures of MMC homologs supplemented with TGF- β_1 and in the cultures of BMMCs grown without TGF- β_1 (Figure 3c). Neither of these cultures had exogenously added IL-10, although both were supplemented with SCF. Mcpt-9 was originally identified in uterine mast cells,⁴⁴ and because in wild-type mice this protease is expressed by intraepithelial mast cells during *T. spiralis* infection, the lack of expression of Mcpt-9 in $\beta_6^{-/-}$ mice may reflect the fact that few of the cells are intraepithelial. The assumption here is that there is a lack of an appropriate environmental signal, although this signal is apparently present in our BMMC cultures. On the other hand, expression of this protease may be down-regulated by other environmental signals in the lamina propria.

A second striking finding that is likely to have a bearing on the differentiation of MMC was the substantial up-regulation of transcription of epithelial SCF in *T. spiralis*-infected $\beta_6^{-/-}$ mice. This may be an additional outcome of decreased epithelial levels of active TGF- β_1 ; TGF- β_1 has been shown to inhibit SCF production by ovarian epithelial cells.⁶¹ In wild-type mice, levels of epithelial SCF transcripts did not change on infection, in agreement with previous findings in mice infected with *Nippostrongylus brasiliensis*.³⁹ SCF is a key regulator of *T. spiralis*-induced intestinal mastocytosis,^{37,38} SCF is produced by the small intestinal epithelium³⁹ and has both systemic and local effects on mast cell development. *In vivo* and *in vitro* studies have shown SCF to have multiple effects on mast cell biology, including directing migration^{48,62} and, in conjunction with IL-3 and IL-4, mast cell growth and proliferation.⁶³

The soluble form of SCF (KL-S) is predominantly generated by proteolytic cleavage from the transmembrane precursor KL-1, but less efficiently from KL-2, in which the

major proteolytic cleavage site is removed by splicing.⁶⁴ Preferential expression of KL-1 rather than KL-2 can be associated with increased production of the soluble form of SCF in epithelial cells.^{61,64} Here, we have found that the KL-1 splice variant of SCF is preferentially expressed in infected $\beta_6^{-/-}$ mice in relation to KL-2, possibly favoring local production of SCF that can be more readily cleaved to its soluble form. Local up-regulation of SCF, diffusing from the epithelium, may contribute to increased proliferation of MMCs in the lamina propria of $\beta_6^{-/-}$ mice. This may partially explain the unusual MMC distribution observed in $\beta_6^{-/-}$ mice, in addition to reduced clearance of MMCs via the epithelium.³⁶

Previous observations have shown that expulsion of the intraepithelial nematode *T. spiralis* is partially dependent on the presence of MMCs⁴⁻⁷ and, specifically, of Mcpt-1.^{8,9} However, the role of this chymase in worm expulsion was demonstrated in a rapid responder BALB/c strain of mouse onto which the Mcpt-1^{-/-} genotype was back-crossed through 10 generations.^{8,9} Our detailed kinetic studies in the 129 strain mice (Figure 7a) indicate that they may be a slower responder phenotype than the BALB/c strain,⁸ with worms persisting until days 23 to 27 after infection. Furthermore, there was still low-level expression of Mcpt-1 in the jejunum, and 4 to 5 $\mu\text{g/ml}$ Mcpt-1 were present in sera of $\beta_6^{-/-}$ mice on days 13 to 17. This is in contrast to Mcpt-1^{-/-} mice in which there is a complete absence of the protease, even though MMCs migrate normally into the epithelium.⁸ The lack of effect on worm expulsion of the reduced numbers of intraepithelial MMCs and decreased expression of Mcpt-1 in $\beta_6^{-/-}$ mice may, therefore, reflect the presence in the mucosa of significant quantities of Mcpt-1, albeit with systemic levels reduced by ~70% compared with controls, and the possible contribution of additional effectors, such as tryptase, from the expanded MMC population in the lamina propria (Figure 2a).

Despite the reduced numbers of intraepithelial MMCs and CD3⁺ T cells in $\beta_6^{-/-}$ mice, there was no significant reduction of levels of IL-4 or IL-13, although there was a trend toward lower levels of IL-13. This implies that the intestinal epithelium itself, or other infiltrating cells, are a source of these cytokines; intraepithelial NK cells in *T. spiralis*-infected mice have recently been identified as a source of IL-13.⁵¹ The consistent expression of TGF- β_1 transcripts in the epithelium of $\beta_6^{+/+}$ and $\beta_6^{-/-}$ mice, as well as the fact that levels are unchanged during infection, is in accordance with our previous observations.³² It is of interest that the epithelial cytokine IL-7 is significantly increased on infection in $\beta_6^{-/-}$ mice. TGF- β_1 and IL-7 have counter-regulatory effects in a number of cell types.⁶⁵⁻⁶⁷ TGF- β_1 is known to down-regulate IL-7 mRNA,⁵⁶ whereas IL-7 can down-regulate TGF- β_1 mRNA.⁶⁸ The function of this cytokine in nematode infection is not known.

Here, we demonstrate that expression of the integrin $\alpha_V\beta_6$ is vital for normal MMC differentiation and protease expression in the gastrointestinal tract and affects epithelial transcription of the cytokines IL-7 and SCF in the epithelium. Our observations strongly support our hypothesis that the integrin $\alpha_V\beta_6$ regulates MMC differenti-

ation in the gastrointestinal epithelium through local activation of TGF- β_1 , which may be spatially or cell-type specific. However, our observations also suggest that $\alpha\beta_6$ on enterocytes has diverse and as yet unknown functions in the epithelium apart from activation of latent TGF- β_1 .

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