Role of STAT6 and Mast Cells in IL-4- and IL-13-Induced Alterations in Murine Intestinal Epithelial Cell Function

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Role of STAT6 and Mast Cells in IL-4- and IL-13-Induced Alterations in Murine Intestinal Epithelial Cell Function

Kathleen B. Madden,* Lucia Whitman,* Carolyn Sullivan,* William C. Gause,† Joseph F. Urban, Jr.,§ Ilidy M. Katona,** Fred D. Finkelman,¶ and Terez Shea-Donohue‡§

Gastrointestinal nematode infections generally invoke a type 2 cytokine response, characterized by the production of IL-4, IL-5, IL-9, and IL-13. Among these cytokines, IL-4 and IL-13 exhibit a functional overlap that can be explained by the sharing of a common receptor or receptor component (IL-4Rα). Binding of IL-4 by either the type 1 or 2 IL-4R, or of IL-13 by the type 2 IL-4R, initiates Jak-dependent tyrosine phosphorylation of the IL-4Rα-chain and the transcription factor, STAT6. In the present study, we investigated: 1) whether IL-13 has effects on intestinal epithelial cells similar to those observed with IL-4, and 2) whether the effects of IL-4 and IL-13 depend on STAT6 signaling and/or mast cells. BALB/c, STAT6−/−, and mast cell-deficient W/W° mice or their +/+ littermates were treated with a long-lasting formulation of recombinant mouse IL-4 (IL-4C) or with IL-13 for seven days. Segments of jejunum were mounted in Ussing chambers to measure mucosal permeability; chloride secretion in response to PGE₂, histamine, 5-hydroxytryptamine, or acetylcholine; and Na⁺-linked glucose absorption. IL-4C and IL-13 increased mucosal permeability, decreased glucose absorption, and decreased chloride secretion in response to 5-hydroxytryptamine. These effects were dependent on STAT6 signaling. Responses to PGE₂ and histamine, which were dependent on mast cells and STAT6, were enhanced by IL-4C, but not by IL-13. The effects of IL-4 and IL-13 on intestinal epithelial cell function may play a critical role in host protection against gastrointestinal nematodes. The Journal of Immunology, 2002, 169: 4417–4422.

The profile of cytokines elicited by an infectious agent orchestrates the host response to the offending pathogen. Gastrointestinal nematode infections, afflicting nearly 1 billion people worldwide (1, 2), generally invoke a type 2 cytokine profile of cytokines elicited by an infectious agent or receptor component (12). The type 1 IL-4R, which includes IL-4Rα-chain and the cytokine receptor common γ-chain, is expressed predominantly by bone marrow-derived cells and binds IL-4, but not IL-13. The type 2 IL-4R, containing IL-4Rα-chain and IL-13Rα-chain, is expressed predominantly by non-bone marrow-derived cells, and binds both IL-4 and IL-13 (13). Binding of IL-4 (by either receptor) or IL-13 (by the type 2 receptor) initiates Jak-dependent tyrosine phosphorylation of IL-4Rα-chain and the transcription factor, STAT6 (14–16). STAT6 is critical for the activation or expression of many IL-4-responsive genes, including class II major histocompatibility molecules, CD23, and the H chain gene for IgE (17–19).

An integral component of the host response to enteric infection is to increase the fluid in the intestinal lumen in an effort to facilitate expulsion, limit access to the surface epithelia, and wash away potential deleterious agents (20). We showed previously that infection with gastrointestinal nematode Heligmosomoides polygyrus decreased glucose absorption and increased fluid secretion in response to the mast cell mediators histamine and PGE₂, effects that were mediated by IL-4 (11). In the current studies, we investigated: 1) whether IL-13 has effects on intestinal epithelial cells similar to those observed with IL-4, and 2) whether the effects of IL-4 and IL-13 depend on mast cells and/or STAT6 signaling.

Materials and Methods

Animals

Male and female 8- to 12-wk-old BALB/c mice and mast cell-deficient W/W° mice and their wild-type (WT) (+/+) littermates were purchased from The Jackson Laboratory (Bar Harbor, ME). STAT6-deficient (STAT6−/−) mice on a BALB/c background were bred at Uniformed Services University of the Health Sciences (Bethesda, MD), and were age and sex matched with controls in all experiments.4

1 This work was supported in part by Uniformed Services University of the Health Sciences Grant R086CD (to K.B.M.), U.S. Department of Agriculture CRIS 1265-32000-060 (to J.F.U.), and National Institutes of Health Grants R01AI35987-06 (to F.D.F.) and R01AI49316-01 (to T.S.-D.). The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Departments of Defense or Agriculture or the Uniformed Services University of the Health Sciences.

2 Address correspondence and reprint requests to Dr. Terez Shea-Donohue, Nutritional Requirements and Function Laboratory, Beltsville Human Nutrition and Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705; and *Division of Immunology, Department of Medicine, University of Cincinnati, Cincinnati, OH 45267

3 Abbreviations used in this paper: MMC, mucosal mast cell; 5-HT, 5-hydroxytryptamine; ACH, acetylcholine; Isc, short circuit current; WT, wild type.

4 These studies were conducted in accordance with the principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, Health and Human Services Publication (National Institutes of Health) 85-23, revised 1996.
Cytokines
Mice were given vehicle or IL-4, as described previously (21), using a long-lasting IL-4 formulation (IL-4C), consisting of 10 μg IL-4 (Peprotech, Rocky Hill, NJ) mixed with 50 μg 11B11, a neutralizing rat IgG1 anti-mouse IL-4 mAb (Veraus, Lebanon, NH). Anti-IL-4 mAb in this formulation is saturated with IL-4 to form complexes that contain a single mAb molecule and two IL-4 molecules. These complexes dissociate in vivo, releasing free IL-4 with a t½ of ~1 day. Because these complexes contain a single IgG molecule, they neither fix complement nor bind more avidly than uncomplexed, monomeric IgG to FcβRs. Furthermore, because the mAb in these complexes blocks the binding of IL-4 to its receptors, complexed IL-4 can only activate its receptor by dissociating from the complex.

BALB/c or STAT6−/− mice were injected i.v. on days 0, 3, and 6 with IL-4C in 0.1 ml normal saline or with an equal volume of normal saline only, and were studied 7 days after the initial injection. Additional groups of mice were injected i.v. with 10 μg rIL-13 (Weyth Research, Cambridge, MA) in 0.2 ml saline, or an equal volume of normal saline on days 0–6, and were studied 7 days after the initial injection.

Ussing chambers
Four 1-cm segments of mucosa were stripped of muscle and mounted in Ussing chambers that exposed 0.126 cm² to 10 ml Krebs’ buffer. Agar-salt bridges and electrodes were used to measure potential difference. Every 50 s, the tissues were short circuited at 1 V (World Precision Instruments DVC 1000 voltage clamp, Sarasota, FL), and the short circuit current (Isc) was monitored continuously. In addition, every 50 s, the clamp voltage was adjusted to 1 V for 10 s to allow calculation of tissue resistance using Ohm’s law.

Following the 15-min equilibrium period, basal Isc, representing the net ion flux at baseline, and tissue resistance, a measure of tissue permeability, were determined. After a second 15-min period, concentration-dependent changes in Isc were determined for the cumulative addition of histamine, PGE2, 5-hydroxytryptamine (5-HT), or acetylcholine (ACH) to the serosal side of the stripped mucosa. After the peak response to the final concentration of each secretagogue was recorded, the Krebs’ buffer on each side of the chamber was replaced, and the tissue was allowed to equilibrate for 30 min. Upon re-equilibration, concentration-dependent changes in Isc were measured in response to the cumulative addition of glucose to the mucosal side. Responses from all tissue segments exposed to glucose from an individual mouse were averaged to yield a mean response per animal.

Solutions and drugs
Krebs’ buffer contained (in mM) 4.74 KCl, 2.54 CaCl2, 18.5 NaCl, 1.19 NaH2PO4, 1.19 MgSO4, and 25.0 NaHCO3 on each side. The tissues were allowed to equilibrate for 15 min in Krebs’ buffer containing 12 mM glucose in the serosal side and 10 mM mannitol on the mucosal side. All drugs were obtained from Sigma-Aldrich (St. Louis, MO), unless stated otherwise. Stock solutions of ACH (1 μM) were prepared in ultrapure water and frozen. PGE2 (1 μM) was dissolved in 100% ethanol and stored at ~70°C. On the day of the experiment, 5-HT and histamine were dissolved in water, and appropriate dilutions of ACH, PGE2, 5-HT, histamine, and glucose were made using distilled water.

Histology
Tissue samples were prepared for visualization of MMC (10). Segments of midjejunum were excised, slit longitudinally, rolled, and placed immediately in Carnoy’s solution and fixed overnight. Tissues were then transferred to 95% ethanol, embedded in paraffin, and sectioned (5 μm). Deparaffinized sections were rehydrated and stained with Alcian blue and Safranin O (Polysciences, Warrington, PA). The numbers of MMC present were enumerated in STAT6−/− mice after 7 days treatment with IL-4C or IL-13 (Fig. 1), demonstrating the STAT6 dependence of this response.

Effects of exogenous IL-4 or IL-13 on ileal epithelial cell absorption in STAT6−/− and WT mice
To assess the effect of IL-13 or IL-4C on substrate-linked sodium absorption, glucose was added to the mucosal (luminal) side of the tissue. IL-4C and IL-13 significantly decreased Isc responses to glucose in WT, but not in STAT6−/− mice (Fig. 2), indicating the STAT6 dependence of this effect.

Effects of IL-4 and IL-13 on mast cell numbers
MMC were enumerated in STAT6−/− and WT mice after 7 days of treatment with IL-4C or IL-13. Untreated STAT6−/− and WT mice had similar numbers of MMC (Fig. 3). MMC were significantly elevated in both WT and STAT6−/− mice treated with IL-4C; however, MMC in IL-4-treated STAT6−/− mice were significantly lower than those in IL-4-treated WT mice (Fig. 3). IL-13 had no effect on MMC numbers in either WT or STAT6−/− mice (Fig. 3).

Cytokine, mast cell, and STAT6 dependence of PGE2 and histamine-induced effects on epithelial cell secretion
In contrast to STAT6-dependent effects of IL-4/IL-13 on intestinal permeability and glucose absorption, IL-4C, but not IL-13, increased glucose absorption in WT (+/+ ) and mast cell-deficient W/W v mice after 7 days treatment with saline or IL-4C (Table II). Isc responses to PGE2 and histamine were significantly enhanced only in the IL-4C-treated +/+ mice, suggesting that these prosecretory effects of IL-4 are mast cell dependent.

Data analysis
Statistical analysis was performed using one-way ANOVA to compare basal Isc and resistance. Cumulative dose responses were compared using multiple ANOVA with post hoc analysis for multiple comparisons. A value of p < 0.05 was considered significant.

Results

Effects of exogenous IL-4 or IL-13 on epithelial cell resistance in STAT6−/− and WT mice
The effects of exogenous IL-13 or IL-4C on intestinal epithelial cell resistance were evaluated in STAT6−/− and WT mice. Resistance, a measure of tissue permeability, was similar in untreated WT and STAT6−/− mice. In contrast, resistance decreased significantly in WT, but not in STAT6−/− mice, treated with IL-4C or IL-13 (Fig. 1), demonstrating the STAT6 dependence of this response.

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FIGURE 1. Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure changes in tissue resistance (an index of epithelial permeability) in WT or STAT6−/− mice after 7 days of treatment with IL-4C (A) or IL-13 (B) (n = 8–12 mice/group). Values are means ± SE; *, p < 0.05 vs WT control.
Because IL-4 induction of intestinal mastocytosis and mast cell degranulation are STAT6 independent (22, 23) (Fig. 3), we expected that the mast cell-dependent mechanism by which IL-4 increases the response to histamine and PGE₂ would also be STAT6 independent. Surprisingly, although exogenous PGE₂ and histamine had similar effects on intestinal epithelial secretory responses when added to the serosal side of intestine from untreated WT and WT vehicle. The tissues were fixed in Carnoy's, sectioned, and stained with Alcian blue and Safranin O. The numbers of MMC present in the lamina propria and mucosa were determined in 50 contiguous high-powered fields. Values are means ± SE; *, $p < 0.05$ vs WT control.

**FIGURE 2.** Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure concentration-dependent changes in $I_{sc}$ in response to glucose in WT or STAT6⁻/⁻ mice after 7 days of treatment with IL-4C (A) or IL-13 (B) ($n = 8–12$ mice/group). Values are means ± SE; *, $p < 0.05$ vs WT control.

**FIGURE 3.** Segments of small intestine were taken from WT or STAT6⁻/⁻ mice after 7 days of treatment with IL-4C or IL-13 ($n = 8–12$ mice/group). The tissues were fixed in Carnoy's, sectioned, and stained with Alcian blue and Safranin O. The numbers of MMC present in the lamina propria and mucosa were determined in 50 contiguous high-powered fields. Values are means ± SE; *, $p < 0.05$ vs WT IL-4C.

### Table I. Changes in epithelial cell secretion in BALB/c (WT) mice treated with IL-4C or IL-13 for 7 days

<table>
<thead>
<tr>
<th></th>
<th>PGE₂</th>
<th>HIST</th>
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<tr>
<td>WT vehicle</td>
<td>52 ± 10</td>
<td>48 ± 9</td>
</tr>
<tr>
<td>WT IL-4C</td>
<td>112 ± 8*</td>
<td>85 ± 8*</td>
</tr>
<tr>
<td>WT IL-13</td>
<td>47 ± 14</td>
<td>44 ± 9</td>
</tr>
</tbody>
</table>

* $I_{sc}$ values are means ± SE expressed as maximum changes in $\mu A/cm^2$; PGE₂ histamine (HIST) = 1 $\mu M$; $n = 3–4$ mice/group. *

$*, p < 0.05$ vs WT vehicle.

### Discussion

Increasing the amount of fluid in the lumen is an integral component of the host response to enteric infection that can facilitate pathogen expulsion, limit pathogen access to the mucosal surface, and dilute pathogen-produced toxins (20). We showed previously that IL-4 mediates a decrease in glucose absorption and an increase in fluid secretion in mice infected with the gastrointestinal nematode parasite *H. polygyrus*, suggesting that this effect of IL-4 may contribute to IL-4-dependent worm expulsion (11). In this study, we compare the effects of in vivo administration of a long-acting formulation of IL-4 with the effects of in vivo administration of the related cytokine, IL-13, on intestinal epithelial cell function, and determine the contribution of STAT6 signaling to these cytokine-induced alterations in intestinal physiology. Because we were concerned about using doses of IL-4 and IL-13 that were biologically equivalent in vivo, we selected dosing regimens that had equal ability to induce the expulsion of the gastrointestinal nematode parasite, *Nippostrongylus brasiliensis*, from SCID or recombinant-activating gene 2-deficient mice (22, 24, 25) (J. Urban, Jr., and F. D. Finkelman, unpublished data).

**Table II. Changes in epithelial cell secretion in +/- and mast cell-deficient (W/Wv) mice treated with IL-4C for 7 days**

<table>
<thead>
<tr>
<th></th>
<th>PGE₂</th>
<th>HIST</th>
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</thead>
<tbody>
<tr>
<td>+/- vehicle</td>
<td>61 ± 13</td>
<td>95 ± 14</td>
</tr>
<tr>
<td>+/- IL-4C</td>
<td>122 ± 33*</td>
<td>187 ± 20*</td>
</tr>
<tr>
<td>W/Wv vehicle</td>
<td>34 ± 8</td>
<td>81 ± 14</td>
</tr>
<tr>
<td>W/Wv IL-4C</td>
<td>49 ± 25</td>
<td>72 ± 23</td>
</tr>
</tbody>
</table>

* $I_{sc}$ values are means ± SE expressed as maximum changes in $\mu A/cm^2$; PGE₂ histamine (HIST) = 0.1 $\mu M$; $n = 3–4$ mice/group. *

$*, p < 0.05$ vs +/- vehicle.
In the current studies, we show that IL-4 and IL-13 induce similar changes in epithelial cell resistance, absorption, and secretion, and that these changes are STAT6 dependent. However, we also demonstrate that IL-4, but not IL-13, increases prosecretory responses to PGE2 and histamine, and that these effects are mast cell dependent. Consistent with this finding, we show that IL-4, but not IL-13, induces intestinal mastocytosis. The ability of IL-4, but not IL-13, to induce intestinal mastocytosis in vivo is consistent with a recent report by Suzuki et al. (26) that IL-4, but not IL-13, promotes in vitro survival and growth of bone marrow-derived mast cells and that the IL-4 effect requires ligation of the type 1 IL-4R (IL-4Rα/H9251/H9253c), which binds IL-4, but not IL-13. Enhancement of the secretory response to PGE2 may have a similar explanation, because we do not see this response in mast cell-deficient mice. Further evidence that differences between IL-4 and IL-13 effects in our model are not explainable by lower relative concentrations of IL-13 than IL-4 comes from our recent observation that IL-13 has a considerably greater stimulatory effect than IL-4, at the same doses that were used in our manuscript, on intestinal smooth muscle contractility (27).

These findings expand those of our previous report (11) in two significant ways. First, our observation that IL-4 and IL-13 induce similar changes in epithelial cell resistance, absorption, and secretion, and that these changes are STAT6 dependent. However, we also demonstrate that IL-4, but not IL-13, increases prosecretory responses to PGE2 and histamine, and that these effects are mast cell dependent. Consistent with this finding, we show that IL-4, but not IL-13, induces intestinal mastocytosis. The ability of IL-4, but not IL-13, to induce intestinal mastocytosis in vivo is consistent with a recent report by Suzuki et al. (26) that IL-4, but not IL-13, promotes in vitro survival and growth of bone marrow-derived mast cells and that the IL-4 effect requires ligation of the type 1 IL-4R (IL-4Rα/H9251/H9253c), which binds IL-4, but not IL-13. Enhancement of the secretory response to PGE2 may have a similar explanation, because we do not see this response in mast cell-deficient mice. Further evidence that differences between IL-4 and IL-13 effects in our model are not explainable by lower relative concentrations of IL-13 than IL-4 comes from our recent observation that IL-13 has a considerably greater stimulatory effect than IL-4, at the same doses that were used in our manuscript, on intestinal smooth muscle contractility (27).

**FIGURE 4.** Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure concentration-dependent changes in $I_\text{sc}$ in response to PGE2 (A) or histamine (B) in WT or STAT6−/− mice after 7 days of treatment with IL-4C ($n = 8–12$ mice/group). Values are means ± SE; *, $p < 0.05$ vs WT control; ♦, $p < 0.05$ vs STAT6−/− control.

**FIGURE 5.** Segments of muscle-free intestinal mucosa were mounted in Ussing chambers to measure concentration-dependent changes in $I_\text{sc}$ in response to 5-HT in WT or STAT6−/− mice after 7 days of treatment with IL-4C (A) or IL-13 (B) ($n = 8–12$ mice/group). Values are means ± SE; *, $p < 0.05$ vs WT control.

we had shown previously that some important effects of IL-4R signaling, such as the induction of mucosal mastocytosis and mast cell degranulation, were STAT6 independent (22).

Our observations confirm and extend some previous reports (e.g., stimulation of mast cell responses by IL-4, but not IL-13) (10) (Fig. 3) and appear to conflict, in part, with others (e.g., that 1) IL-4 treatment of the human intestinal cell line T84 in vitro inhibits Cl− secretion (28, 29), and 2) IL-4/IL-13 induce a STAT6-independent, phosphatidylinositol 3-kinase pathway-dependent, increased transepithelial permeability in vitro in the human T84 intestinal cell line (30)). This apparent conflict may be explained by the in vivo administration of the cytokines in our study, as well as by inherent differences in the function of homogeneous intestinal epithelial cell lines vs excised intestinal mucosae with its intact neural circuitry. However, it is of interest to note that two

**FIGURE 6.** Schematic depiction of the roles of STAT6, IL-4, and/or IL-13 in murine intestinal epithelial cell function and MMC hyperplasia.
other recent reports have shown 1) a dose-dependent decrease in the resistance of rat glomerular visceral epithelial cells in vitro to response to IL-4 or IL-13 (31), and 2) decreased Na⁺ absorption and increased Cl⁻ secretion in vitro to response to IL-4 treatment of human bronchial epithelial cells (32). Results of these studies suggest that the effects of IL-4 and IL-13 on epithelial cell function may not be limited to the gastrointestinal tract, but rather may represent a broader mechanism of immunoregulation at epithelial cell surfaces.

More importantly, our observations indicate that the Th2 cytokines, particularly IL-4 and IL-13, change intestinal epithelial function through multiple effects that additively or synergistically interact to shift the balance of ion and fluid flow toward the gut lumen, creating the increase in luminal fluid that may protect the host against pathogens. The complexity of these interactions is illustrated by the IL-4 effects on intestinal responsiveness to PGE₂ and histamine, which must have at least two components. The mast cell dependence of this effect of IL-4 and its failure to be induced by IL-13 (which does not stimulate mast cells) suggest that it requires IL-4 stimulation of mastocytosis. However, IL-4 induction of mast cell hyperplasia and mast cell degranulation (as measured by an increase in serum levels of mouse mast cell protease) (22, 23) (Fig. 3) is STAT6 independent, while IL-4 enhancement of the proteocytotoxic effects of PGE₂ and histamine is STAT6 dependent. It remains to be determined whether STAT6 signaling is required to induce mast cells to release specific mediators that promote increased responsiveness to PGE₂ and histamine, or whether there is a separate, STAT6-dependent effect of IL-4 on intestinal epithelial cells that acts with a STAT6-independent mast cell effect to increase intestinal epithelial responsiveness. In support of the latter possibility, IL-4 has been shown to act through a STAT6-dependent mechanism to: 1) increase responsiveness to platelet-activating factor, histamine, 5-HT, and leukotriene C₄ in an anaphylaxis model (33, 34); 2) induce increased expression of a receptor for cysteinyl leukotrienes (35); and 3) promote mast cell-dependent expulsion of Trichinella spiralis by infected mice through an effect on non-bone marrow-derived cells (24).

This difference in the effects of IL-4 and IL-13 on mast cells and mast cell-dependent epithelial function probably has consequences for host responses to intestinal worm infection and may explain differences in the relative importance of IL-4 and IL-13 in host protection against different parasites. Mice infected with N. brasiliensis do not require mast cells for parasite expulsion and exhibit a stronger dependence on IL-13 than IL-4 for worm expulsion (22). This greater dependence on IL-13 probably reflects either greater production of IL-13 than IL-4 by infected mice or increased potency of IL-13 vs IL-4 in the induction of a host-protective effect, because treatment of N. brasiliensis-infected mice with IL-4 induces worm expulsion in the absence of IL-13. In contrast, the mast cell-dependent expulsion of T. spiralis is more dependent on IL-4 than on IL-13, particularly during a second infection with this parasite (23). Thus, the secretion of both IL-4 and IL-13 during worm infections and the multiple mechanisms by which these cytokines promote changes in intestinal epithelial cell function appear to extend the ability of the Th2 cytokine response to protect against a spectrum of intestinal nematode parasites.

Finally, our observations demonstrate that not all effects of IL-4 and IL-13 on intestinal epithelial cells are prosecretory. Although 5-HT normally increases intestinal epithelial cell secretion, IL-4 and IL-13 inhibit this effect through a STAT6-dependent process. Furthermore, treatment of STAT6⁻ /⁻ and WT mice with IL-4 or IL-13 in vivo had no effect on the secretory response to ACH.

Thus, exposure to IL-4 and IL-13 shifts the relative importance of different mediators in regulating intestinal epithelial ion flow as well as the effects of specific mediators on this process. The role of inhibitory effects of IL-4/IL-13 on intestinal epithelial function, and the mechanisms by which inhibitory and stimulatory effects interact during parasite infection, remain to be determined.

**Acknowledgments**

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**References**


4422 STAT6 DEPENDENCE OF CYTOKINE EFFECTS ON INTESTINAL EPITHELIAL FUNCTION EFFECTS