Downregulation of the ubiquitin-proteasome system in normal colonic macrophages and reinduction in inflammatory bowel disease

Hetzenecker, A M; Seidl, M C; Kosovac, K; Herfarth, H; Kellermeyer, S; Obermeier, F; Falk, W; Schoelmerich, J; Hausmann, M; Rogler, G

Abstract: BACKGROUND: In normal mucosa, intestinal lamina propria macrophages (IMACs) maintain tolerance against food antigens and the commensal bacterial flora. Several mechanisms have been identified that mediate tolerance. The ubiquitin-proteasome system (UPS) is a large multiprotein complex that degrades cellular proteins. As the UPS may modulate immune functions of IMACs, we performed a detailed investigation of UPS expression and function under normal conditions and in cells derived from patients suffering from inflammatory bowel disease (IBD). METHODS: IMACs were isolated from intestinal mucosa. mRNA expression of macrophages differentiated in vitro (i.v. MACs) and IMACs was compared by Affymetrix® oligonucleotide arrays. Quantitative Taqman-PCR was performed on five exemplary proteasomal and five ubiquitinylation genes each. Proteins were analyzed by immunohistochemistry and Western blotting. Proteasome function was assessed by a fluorimetric test. RESULTS: Affymetrix analysis showed downregulation of mRNA expression of almost all represented proteasomal and of 22 ubiquitination-associated genes in IMACs as compared to i.v. MACs and monocytes. By quantitative PCR, up to tenfold higher mRNA expression of 10 exemplary genes of the UPS (UBE2A, UBE2D2, UBE2L6, USP14, UBB and ATPase2, 2, 5, 2i/MECL-1, 5i/LMP7) was demonstrated in i.v. MACs as compared to IMACs. Immunohistochemistry and Western blots confirmed these findings in intestinal mucosa of controls and patients suffering from diverticulitis. In contrast, a significant increase in protein amounts was found in mucosa of patients with IBD. CONCLUSION: Reduced expression of subunits of the UPS in IMACs of normal mucosa supports the concept of the presence of a nonreactive, anergic macrophage phenotype in the gut under normal conditions. Reinduction in IMACs of IBD mucosa reflects activated IMACs which can present antigenic peptides and thus support inflammation.

DOI: https://doi.org/10.1159/000336353

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-76217
Accepted Version

Originally published at:
Hetzenecker, A M; Seidl, M C; Kosovac, K; Herfarth, H; Kellermeyer, S; Obermeier, F; Falk, W; Schoelmerich, J; Hausmann, M; Rogler, G (2012). Downregulation of the ubiquitin-proteasome system in normal colonic macrophages and reinduction in inflammatory bowel disease. Digestion, 86(1):34-47.
DOI: https://doi.org/10.1159/000336353
Downregulation of the ubiquitin-proteasome system in normal colonic macrophages and re-induction in inflammatory bowel disease


Point by point reply to the reviewer

On the basis of a comprehensive gene expression analysis, Hetzenecker and co-workers report their results on the ubiquitin-proteasome system (UPS) in intestinal lamina propria macrophages. The authors demonstrate that gene expression levels of subunits of the UPS are lower in IMACs compared to in vitro generated macrophages. Furthermore, Hetzenecker et al. show an increased gene expression of the same components of the UPS in IMACs isolated from the mucosa of IBD patients when compared to IMACs isolated from control patients. The authors confirm these findings also on a protein level using immunohistochemistry and Western blotting.

The reported low expression levels of the ubiquitinylation machinery and proteasome in IMACs, indeed indicates that the proteasomal function in these cells is decreased and supports the concept of anergic lamina propria macrophages in the healthy mucosa.

To conclude, although it is mainly a descriptive study, which may greatly benefit from additional functional assays, the reported findings are novel and may lead to further investigations in the field.

Major recommendation.

The authors perform gene expression analysis on mRNA from IMACs isolated by antiCD33 MACS. Due to the presence of CD33 also on monocytes, a contamination of the purified IMAC population by invading monocytes cannot be excluded. This becomes mostly evident when IMACs are isolated from affected IBD mucosa, as is mentioned in the manuscript. By showing that ubiquitinylation genes and the proteasomal machinery is downregulated in circulating monocytes of IBD patients, Hetzenecker et al. conclude that the increased UPS expression found in the affected mucosa is likely to be a local event. To further strengthen this argument, the authors wish to provide the reader with information on the purity of the MACS sorted IMAC populations from normal compared to IBD mucosa. By the use of flow cytometry and additional markers than CD33, the cellular composition of the macrophage populations after MACS purification could be determined. This would allow to assess the proportion of contaminating cells.

Minor comments.

For the general understanding, the authors wish to include a more detailed introduction of the UPS in the context of intestinal inflammation.

(i) Figures.
Fig. 1B shows signal arbitrary units of 4 selected ubiquitinylation genes (UBB, USP14, UBE2A, UBE2D), while in the figure caption is mentioned: ?? five selected ubiquitinylation genes (B).?

Additional to the p-values in Figs. 2-5 and 7, it is important to know which statistical test was used and how many patients per group were included in the calculation. Furthermore, in Figs. 3-5 asterisks mark significant differences without providing further information to which other values in the corresponding graphs these differences correlate. The authors should provide this information.

(ii) Materials and Methods.

The section ?Generation of i.v.MACs? is too undefined: Which method was used for the isolation of monocytes and how were they identified and checked for purity? The relative composition of the two major subsets of blood monocytes (CD14+CD16- vs. CD14-CD16+) may greatly vary depending on the used isolation protocol (MACS?, FACS?, gradient centrifugation?).

The section ?Proteasome function? lacks information on the normalisation of the assay. Was the proteasome activity normalised to mucosal tissue weight? Information on that would help to understand the meaning of the Y-axis in Fig. 7 (ng/ul proteasome activity). If the assay was normalised to tissue weight, the authors may wish to reconsider their conclusion on p. 19 line 6: ?? proteasome and ubiquitinylation function was induced in IMACs ...?. Based on a missing parameter which allows to directly correlate proteasomal function to the amount of IMACs present in the tissue, proteasome activity can not be correlated to a single cell population rather to the whole cellular tissue content that was analysed.

(iii) Discussion

In the discussion (p. 20 line 2) the authors mention that the ubiquitinylation machinery and expression of proteosomal components is downregulated in IMACs as compared to monocytes and i.v.MACs. However, in Fig. 5 Hetzenecker et al. show a trend for higher UPS gene expression in IMACs compared to monocytes. The authors may wish to clarify this contradiction.

(iii) Typos.

p. 12 line 18: ?form? -> from
Fig. 3A: The asterisk defining p>0.01 in the 5i/LMP7 analysis marks ?UC remission? -> should mark ?UC active disease?
Suppl. Table 2 ?Primer sequences? -> Suppl. Table 3 ?Primer sequences?
Suppl. Table 1B -> ? mRNA isolation for quantitative PCR from 14 patients.