

Intestinal IgA-positive plasma cells are highly sensitive indicators of alloreaction early after allogeneic transplantation and associate with both graft-versus-host disease and relapse-related mortality

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Supplemental files:

Supplemental Methods:

IgA staining:

IgA⁺ plasma cells in the investigated biopsies were stained by immunohistochemistry (Polyclonal Rabbit Anti-Human IgA, Code-Nr. A 0262; Dako Denmark A/S, Glostrup, DK), supported by a software-controlled slide stainer (VENTANA BenchMark ULTRA; Ventana Medical Systems Inc., Tucson, USA). After antigen-retrieval and binding of secondary and tertiary antibodies to the primary antibody, the final colour reaction of diaminobenzidine was catalysed by the horseradish peroxidase conjugated to the tertiary antibody using the OptiView DAB IHC Detection Kit (Roche diagnostics, Mannheim, Germany) resulting in a brownish appearance in the Lamina propria and the involved epithelium. L. muscularis mucosa as well as artefacts and gaps were excluded. An examination area of at least 0.3 mm² per sample was determined and the cell concentration was calculated as the amount of IgA⁺ plasma cells per mm². IgA⁺ cells were almost exclusively located in the subepithelial lamina propria.

IgA serum levels:

In 108 pts, serum samples had been drawn within +/-7 days of master biopsies thus allowing pairwise assessment of IgA serum levels and IgA⁺ plasma cells. Serum samples were stored at -80°C until analysis. IgA serum levels were quantified in a DIN ISO 15189 accredited clinical laboratory using an immunoturbidimetric assay (Roche Tina-quant IgA Gen.2) on an automated clinical chemistry analyzer (Roche cobas pro, Grenzach Whylen, Germany). Sample preparation and measurements were performed according to the manufacturer's instructions. Sample concentrations above 800 mg/dl were re-measured using the automated rerun function after 1:8 dilution. Likewise, sample concentrations below 50 mg/dl were re-measured using the automated rerun function with 10-fold increased sample volume.

Supplemental tables:

Supplemental Table 1:

No impact of the site of biopsy on GvHD dependent suppression. Number of biopsies and mean (+/-SE) of IgA+ plasma cells are shown

Site	Histological GvHD	n	IgA+ plasma cells/mm² (SE)
Upper GI	Not at all	85	139.8 (13.3)
	Lerner 1-4	68	102.7 (13.7)
Lower GI	Not at all	115	122.7 (9.0)
	Lerner 1-4	129	97.8 (9.3)

Supplemental Figures:

Supplemental Figure 1: Correlation of IgA serum levels and IgA+ intestinal plasma cells: Samples from pts with higher GvHD stages cluster in the low plasma cell/low IgA area. A total of 108 serum/biopsy pairs were analyzed ($r=0.32$, $p=0.00$)

