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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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Running heads: Intestinal IgA positive cells indicate GvH and GvL

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Abstract:

Intestinal IgA is strongly involved in microbiota homeostasis. Since microbiota disruption is a major risk factor of acute GvHD, we addressed the kinetics of intestinal IgA-positive (IgA^+) plasma cells by immunohistology in a series of 430 intestinal biopsies obtained at a median of 1,5 months after allogeneic stem cell transplantation from 115 patients (pts) at our center. IgA^+ plasma cells were located in the subepithelial lamina propria and suppressed in the presence of histological aGvHD (GvHD lerner stage 0: 131+/- 8 IgA^+ plasma cells/mm^2; stage 1-2: 108 +/-8 IgA^+ plasma cells/ mm^2; stage 3-4: 89+/-16 IgA^+ plasma cells/ mm^2, p 0.004). Overall, pts with IgA^+ plasma cells below median had an increased treatment related mortality (p = 0.04). Time courses suggested a gradual recovery of IgA^+ plasma cells after day 100 in the absence but not in the presence of GvHD. Vice versa IgA^+ plasma cells above median early after SCT were predictive of relapse and relapse related mortality (RRM): Pts with low IgA^+ cells had a 15% RRM at 2 and at 5 yrs, while pts with high IgA^+ cells had a 31% RRM at 2 years and more than 46% at 5 years; multivariate analysis indicated high IgA^+ plasma cells in biopsies (HR 2.7 (95% CI 1.04-7.00) as independent predictors of RRM, whereas Lerner stage and disease stage themselves did not affect RRM. In contrast, IgA serum levels at the time of biopsy were not predictive for RRM. In summary, our data indicate that IgA^+ cells are highly sensitive indicators of alloreaction early after allo-SCT showing association with TRM but also allowing prediction of relapse independently from the presence of overt GvHD.

Introduction:

Graft-versus-host disease (GvHD) and graft-versus-leukemia reactions (GvL) are major determinants of outcome following allogeneic stem cell transplantation (allo-SCT) and show a broad overlap thus frequently preventing clear clinical separation of beneficial GvL and deleterious GvH effects.[1] Acute GvHD strongly affects the intestinal (GI) tract, and microbiota colonizing the GI tract have been identified as major modifiers of both, normal and pathologic immune reactions such as GvHD. [2-4] Numerous immune cells including alloreactive T cells, regulatory T cells and innate lymphoid cells are involved in immune regulation of GvHD but also maintenance of immunological homeostasis in the GI tract [1, 5] Likewise, intestinal plasma cells producing mainly IgA antibodies play a pivotal role in this context: They are strongly reduced in germfree mice,[6] induced in Peyers patches and secondary lymphoid organs and disseminate to the lamina propria of the whole GI tract. They are the main producers of secretory IgA which plays a central role in defense against pathogens but also in maintaining coexistence with commensal bacteria, and flow cytometry of IgA coated bacteria in the GI lumen revealed coating of a wide range of commensals. [7-9]

Intestinal IgA^+ plasma cells have not been in the focus of GvHD pathophysiology so far, only a few early studies reported suppression of intestinal plasma cells in patients (pts) dying from GvHD.[10] More is known about serum IgA levels and B cell deficiency in general in relation to GvHD: Delayed and disturbed B cell recovery is a hallmark of acute (aGvHD) and chronic GvHD (cGvHD) [11] and both, decrease and low IgA levels and B cell deficiency associate with history of aGvHD and severity of cGvHD.[12] Impaired reconstitution of IgA levels is among the most sensitive indicator of the presence of cGvHD[12] , and immunoglobulin deficiency can persist up to 5 years and more in pts with cGvHD.[13]

Based on the potential role of IgA in microbiota regulation and the association of GvHD and microbiota damage we decided to perform a large retrospective analysis of intestinal IgA^+ plasma cells in a series of biopsies obtained from pts after allo-SCT:

Our study revealed a prolonged suppression and deficiency of IgA^+ plasma cells in pts with GvHD and revealed a so far not reported association of increased IgA^+ plasma cells with a highly increased relapse related mortality (RRM) suggesting plasma cells as a sensitive target of alloreaction involved in GvL even beyond overt GvHD.

Methods:

Patients:

Patient characteristics are given in table 1 and represent a typical adult allo-SCT population. All pts gave informed consent to use urine and serum samples as well as biopsy sections for additional analysis including IgA serum titers and IgA staining of biopsies. The study was approved by the local ethical review board of the University of Regensburg (Approval number 09/059).

Biopsies:

A total of 430 biopsies from 128 pts were analyzed. 36 pts had 2 biopsies, 61 pts had 3 and more biopsies, from different sites of the GI tract and at different time points. 34 biopsies from 13 pts were obtained prior to allo-SCT to rule out other GI diseases and served as pretransplant controls. The median time to biopsies was 1.5 months after allo-SCT (range 0.4 – 67 months), 85% of biopsies were obtained within a time period of less than 6 months after transplantation.

To avoid any bias by multiple biopsies in pts with more severe courses, we defined 1 most relevant (=master) biopsy per pt for survival and outcome analysis: In pts with only one biopsy this corresponded to the only available one. For pts with multiple biopsy, the biopsy obtained at onset of GvHD was selected, and for pts without GvHD, the first biopsy after allo-SCT was used. Selection was independent of biopsy location and the number of allo-SCTs. Outcome was always analyzed in relation to the biopsy of the respective transplant.

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). The detailed protocol is given in supplemental files.

Factors influencing IgA^+ plasma cells

To analyze the impact of time after allo-SCT, biopsies were grouped according to the time period of biopsy into biopsies pretransplant, biopsies obtained until d100 and

biopsies obtained later than d100 after allo-SCT. To assess the impact of microbiota, we used urinary indoxylsulfate levels (µmol/mmol creatinine) as previously described[14] which were available within 1 week in relation to biopsies for 197 pts. For each biopsy, we recorded the concomitant treatment with corticosteroids > 20mg/d and the use of Rituxan.

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, details see Suppl file).

Statistical analysis:

Clinical data as well as data from histopathological analyses including Lerner stage and IgA^+ cells/mm^2 were collected in a SPSS database (Version 26, IBM New York, USA). For comparisons of mean IgA^+ plasma cells non-parametric Wilcoxon tests were used. For survival analysis using Kaplan Meier, Cox Regression and competing risk assessment, master biopsies were selected.

Results:

Morphology and distribution in the GI tract

IgA^+ plasma cells could be easily identified by immunohistochemistry in the lamina propria and were in close contact to the epithelial lines (Fig 1). When we compared the amount of plasma cells in relation to the site of biopsies, there was no significant difference between the upper and the lower GI tract (upper GI tract n = 153: 124.5 (9.4) IgA^+ plasma cells/ mm^2 vs lower GI tract n= 244: 110.0 (6.4) IgA^+ plasma cells/ mm^2.

Low intestinal IgA^+ plasma cell numbers associate with aGvHD and treatment related mortality

Next, we addressed the impact of aGvHD on the presence of the IgA^+ plasma cells: IgA^+ plasma cells were highest in 200 pts with Lerner stage 0, and gradually decreased with more severe GvHD (table 2). For the whole set of biopsies obtained after allo-SCT, GvHD dependent effects were observed both in the upper and in the lower GI tract (Suppl. Table 1).

When we analyzed patients according to organ involvement and Lerner stage, suppression of IgA^+ plasma cells was only mild in pts with exclusive skin or liver GvHD but highly pronounced and significant in pts with overt gastrointestinal involvement.

A total of 32 (27.8%) of pts died from treatment related complications such as GvHD +/- infections. In line with a stronger suppression of plasma cells in pts with more severe GvHD, patients with IgA^+ plasma cells below median after allo-SCT experienced an increased TRM (log rank 0.04, figure 2). In a multivariate cox regression analysis of TRM, higher Lerner stage (HR 3.8 – 95% CI 1.6 -9.6) predicted TRM and higher IgA^+ cell numbers were protective (HR 0.34, 95% CI 0.14 – 0.83), whereas underlying disease, age, stage and donor type did not have significant impact (data not shown).

Suppression of IgA$^+$ plasma cells by GvHD is independent of microbiota damage, corticosteroid use or use of B cell depleting agents at the time of biopsy and from time after allo- SCT

When we grouped biopsies according to the time after allo-SCT, 41 biopsies obtained prior to allo-SCT contained 139.0 (23.7) IgA$^+$ plasma cells/ mm^2 whereas biopsies obtained until day 100 after allo-SCT showed 107.6 (7.2) IgA$^+$ plasma cells (n=244, p 0.08 vs pretransplant). In biopsies obtained beyond day 100 IgA$^+$ plasma cells started to recover again (n=153; 125.7 (7.9) IgA$^+$ plasma cells; p 0.01 vs before d100). GvHD dependent suppression, however, was observed for both time intervals: In 233 biopsies obtained before d100, IgA$^+$ plasma cells/mm^2 were 121.9 (10.4) in biopsies without histological GvHD and 87.8. (9.2) in biopsies with GvHD (p 0.001), in 163 biopsies obtained after d100, mean IgA$^+$ plasma cells/mm^2 were 140.6 (11.0) in the absence of GvHD and 110.9 (11.9) in the presence of GvHD (p 0.03). Analysis of urinary indoxylsulfate (IS) levels (indicating presence of commensal microbiota in the gut), was available for 197 biopsies: IS levels were 97.6 (10.9) µmol/mmol creatinine for 87 biopsies with IgA below median and 87.2 (9.2) µmol/mmol creatinine for 107 biopsies with IgA above median (difference not significant). As corticosteroid dosage and use of B cell depleting agents like rituximab might affect IgA$^+$ plasma cells, we also analyzed prednisolone usage above 20mg/d at the time of biopsy and did not observe significant differences (data not shown). Similarly, mean IgA plasma cells were 88.2 (+/- 18.0) IgA+ plasma cells/mm^2 in 14 pts receiving rituximab prior to SCT versus 95.7 +/- 9.3 IgA+ plasma cells/mm^2 in pts not recveiving rituximab (ns). After SCT, only 4 pts received rituximab prior to biopsies for treatment of high EBV serum copy numbers to prevent EBV lymphoma which had no impact on mean IgA plasma cells in the biopsies (104.8+/- 58.0 IgA+ plasma cells/mm^2 as compared to 93.9 +/- 8.0 IgA+ plasma cells/mm^{2}the remaining pts (ns).
Thus, in a multivariate binary logistic regression, only advanced Lerner stage (OR 0.51 -95% CI 0.26 – 0.93) but not prednisolone dose, microbiota damage, time interval after allo-SCT, age and site of biopsy were of significant impact (data not shown).

Intestinal IgA$^+$ plasma cells associate with relapse even in the absence of GvHD

Presence of high IgA$^+$ plasma cell numbers in the GI tract on the other side associated with relapse and relapse related mortality (RRM). Whereas only 15% of 57 pts with IgA$^+$ plasma cells below median at the time of biopsy died from relapse in long term

follow up, 31% of 58 pts with high IgA^+ plasma cell content (above median) died at 2 years and 46% at 5 years after allo-SCT (figure 3. p = 0.01). When we included 6 additional patients with relapse but alive at the end of the observational period and assessed relapse incidence in general, we observed a comparable association: Only 11.5% of patients with low IgA plasma cells relapsed in contrast to 41% of patients with high IgA plasma cells (p 0.001 in log rank).

These associations were predominantly observed in pts without evidence of major GvHD in their biopsies (Lerner 0-1, log rank p = 0.03). In multivariate Cox regression only IgA^+ plasma cells, but not Lerner stage, disease stage at the time of transplant or age predicted relapse related mortality, suggesting a prognostic significance of IgA^+ plasma cell numbers independently from presence of overt GvHD (table 3).

To rule out an impact of underlying diseases we performed a separate analysis for myeloid versus lymphoid malignancies: in both subgroups IgA^+ plasma cells predicted RRM: In myeloid malignancies, RRM at 5 yrs was 16% versus 49% in pts with low and high IgA^+ plasma cell numbers, respectively, whereas in lymphoid malignancies RRM was 0% and 53%, respectively. Both results strongly support a disease independent observation.

Finally, we performed a competing risk analysis to rule out mutual overlap between GvHD and GvL effects. IgA^+ plasma cells were independent predictors of both, TRM (low IgA^+ plasma cell numbers) and RRM (high IgA^+ plasma cell numbers) (figure 4).

IgA serum levels show weak correlation with intestinal IgA^+ plasma cells and only add to prediction of TRM

As serum IgA might allow more rapid assessment of prognostic IgA^+ plasma cell deficiency, we addressed whether IgA serum levels at the time of master biopsies reflected intestinal IgA^+ plasma cell content. There was some correlation between IgA serum levels and the amount of IgA^+ plasma cells (r = 0.332, p 0.000) in 108 available serum/biopsy pairs (Suppl figure 1). Patients with higher Lerner stages showed a tendency to have lower IgA serum levels, as pts with Lerner 0 (n=48) had 82.0 (SEM 9.3), pts with Lerner 1,2 (n=44) had 84.7 (SEM 13.4) and pts with Lerner 3,4 (n=16) had 48.1 (SEM 7.1) g/l IgA (Lerner 3,4 vs 0 p 0.03, all other ns).

When we compared IgA^+ plasma cells in the GI tract and IgA serum levels (above/below median) in Kaplan Meier analyses regarding TRM and RRM, low serum IgA levels were weakly associated with higher TRM but showed no correlation with RRM as observed for IgA^+ plasma cells (data not shown).

Discussion:

Our study describes for the first time the association of intestinal IgA$^+$ plasma cells with outcome following allo-SCT in a large series of pts. Although it is well known, that the majority of IgA-producing plasma cells reside in the intestinal tract and contribute substantially to control of microbial inflammation[6,15], only a few studies so far have addressed their changes in the setting of allo-SCT and GvHD. These papers addressed IgA bearing cells in human autopsies and showed that loss of these cells is part of a general immune cell loss during the course of aGvHD of the intestinal tract.[10,16]. In the same line, we recently described B cells in the bone marrow and spleen as being the most sensitive indicators of aGvHD and that their loss could be prevented or reversed by treatment with donor-derived regulatory T cells.[17] Prolonged secondary IgA deficiency in the serum is a hallmark of impaired systemic immunoreconstitution following allo-SCT and it is aggravated in pts with cGvHD.[12,13,19] Although the majority of serum IgA is produced by plasma cells residing in the bone marrow , intestinal plasma cells can also significantly contribute to systemic IgA levels. Thus low serum IgA could indicate damage to plasma cells, both in the marrow and the intestinal tract[18], which is in line with the moderate correlation between serum IgA and intestinal IgA$^+$ plasma cells seen in our study.

Our systematic analyses confirmed a clear suppression of IgA$^+$ plasma cells in intestinal aGvHD and a subsequent association of this suppression with an increase in TRM. Suppression was most prominent in the first 100 days after allo-SCT. Thereafter plasma cell numbers started to recover in the absence of aGvHD, whereas a stronger suppression persisted in patients with severe aGvHD throughout the study. Besides time after allo-SCT, we tried to identify other confounding factors suppressing IgA$^+$ plasma cells, but neither microbiota damage at the time of biopsy, as indicated by urinary IS levels, nor concomitant immunosuppression with high dose corticosteroids or B cell depleting agents like rituximab nor the site of biopsy affected the strong impact of histological GvHD. Interestingly, suppression of intestinal IgA$^+$ plasma cells was minor if clinical GvHD involved only organs such as skin and/or liver, suggesting local mechanisms being active in GI- GvHD and damaging plasma cells residing in that organ.

A yet unanswered question is the origin of intestinal IgA$^+$ plasma cells at different time points following allo-SCT. Based on immunoglobulin recovery in general and half-life

of specific antibodies (like anti-HbS),[20] donor plasma cells are starting to take over immunoglobulin production 12 to 18 months after allo-SCT. However, reports on intestinal plasma cells, e.g. after small bowel transplantation, suggest that individual recipient-derived plasma cells may even persist for years.[21] Onset of regeneration of plasma cells beyond day 100 may be in line with these kinetics, however detailed studies on plasma cell chimerism both in patients and in experimental murine models are required in the future. This should also contribute to decipher the underlying mechanism of plasma cell damage. Whereas our study suggests direct elimination of intestinal IgA+ plasma cells by alloreactive T cells, actual and ongoing murine and human studies by our group indicate a more general and broader damage of the B cell compartment in GvHD with an arrest of B cell and plasma cell maturation which might indicate damage to the B cell niches independent from the actual chimerism of B cell effectors. Whether these mechanisms contribute to the suppression of plasma cells observed in our study needs to be analyzed in future studies.

The differential recovery of IgA+ plasma cells already highlights the high sensitivity of plasma cells to allo-reaction in the setting of GvHD. The unexpected finding of increased plasma cells early after allo-SCT as a sensitive predictor of relapse and RRM, is in line with this sensitivity, as our observations suggest that decreased elimination of plasma cells may reflect an impaired alloreaction against recipient hematopoietic cells, which is active also in the absence of overt clinical GvHD. This GvHD independent alloreaction is currently thought to be the most broadly active mechanism of GvL effects mediated by a graft-versus-host hematopoiesis reaction.[22] Again, detailed analysis of chimerism of plasma cells will help to sharpen this hypothesis.

So far, appropriate broad biomarkers predicting relapse are missing. Antigen specific T cells have been reported for WT1 antigen positive leukemia cells,[23] and analysis of MRD where available is another example of specific biomarkers. Direct analysis of the extent of alloreaction is only possible on the level of chimerism,[24] thus assessment of intestinal plasma cells if confirmed as a predictor of relapse might be a useful indicator and help to guide preemptive strategies such as donor lymphocyte infusions (DLI). As the easily accessible serum IgA levels have not been analyzed in this context so far, we compared the prognostic significance of serum IgA and intestinal IgA^+ plasma cells. In spite of some positive correlation, analysis of intestinal plasma cells seemed to be a stronger indicator of alloreaction as compared to assessment of systemic IgA levels. Secretion of IgA, e.g. in the form of fecal IgA, might be an alternative approach to

assess intestinal plasma cell activity but has not been addressed in pts after allo-SCT so far.[25]

In summary, our study reports a strong association of intestinal IgA+ plasma cells with alloreaction in the setting of GvHD which has impact on both, GvHD and GvL.

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Legends for Tables

Table 1: Patient and transplant characteristics:

115 pts after allo-SCT were analyzed, absolute numbers and %/range are shown

Underlying diseases: AML = acute myelogenous leukemia, ALL = acute lymphoblastic leukemia, MDS = myelodysplastic syndrome, MPS = myeloproliferative syndromes (OMF = osteomyelofibrosis, CML = chronic myelogenous leukemia), NHL = Non Hodgkins Lymphoma (Hg, lg = high and low grade)

Donor: URD = unrelated donor, haploid. = haploidentical

Stem cell source: PBSC = mobilized peripheral blood stem cells

Table 2: GvHD dependent suppression of IgA^+ plasma cells

Differences between Lerner 0 and Lerner stage 1/2 were significant with p 0.009, differences between Lerner stage 1,2 and stage 3,4 were significant with p 0.02.

Table 3: Cox regression analysis for RRM: High IgA^+ plasma cells is the only independent predictor. 95% CI = 95% confidence interval.

Table 1: Patient and transplant characteristics

Gender f/m	45/70	39/61%
Age: Median/range	56 yrs	17-70yrs
Underlying disease:		
AML	60	54%
ALL	4	3%
MDS	13	11%
MPS (OMF, CML)	13	11%
Myeloma	9	8%
LgNHL	3	2%
HgNHL	9	8%
Non malignant	4	3%
Stage at Tx		
Early	28	25%
Intermed.	52	45%
advanced	35	30%
Conditioning		
Standard	102	89%
Reduced Intensity	13	11%
Number of SCTs:		
1	99	86%
2 or 3	16	14%
Donor:		
Sibling	34	29%
URD	72	63%
Haploid.	9	8%
Stem cell source:		
PBSC	105	91%
marrow	10	9%

Table 2: GvHD dependent suppression of IgA$^+$ plasma cells:

Histological GvHD	n	IgA+ plasma cells/ mm^2 (SE)
No GvHD at all	200	129.5 (7.7)
Lerner stage 1/2	143	103.7 (8.2) **
Lerner stage 3/4	54	91.8 (17.3) *

Table 3: Cox regression analysis of factors associated with increased relapse related mortality

Factor	HR	P	95% CI
Lerner Grp	1.59	Ns	0.4– 5.9
Age at alloSCT	1.98	Ns	0.7 – 6.0
Advanced stage	2.18	ns	0.7 – 8.5
Underlying disease	0.66	ns	0.2 – 2.4
IgA+ plasma cells > median	3.33	0.03	1.1 – 10.1

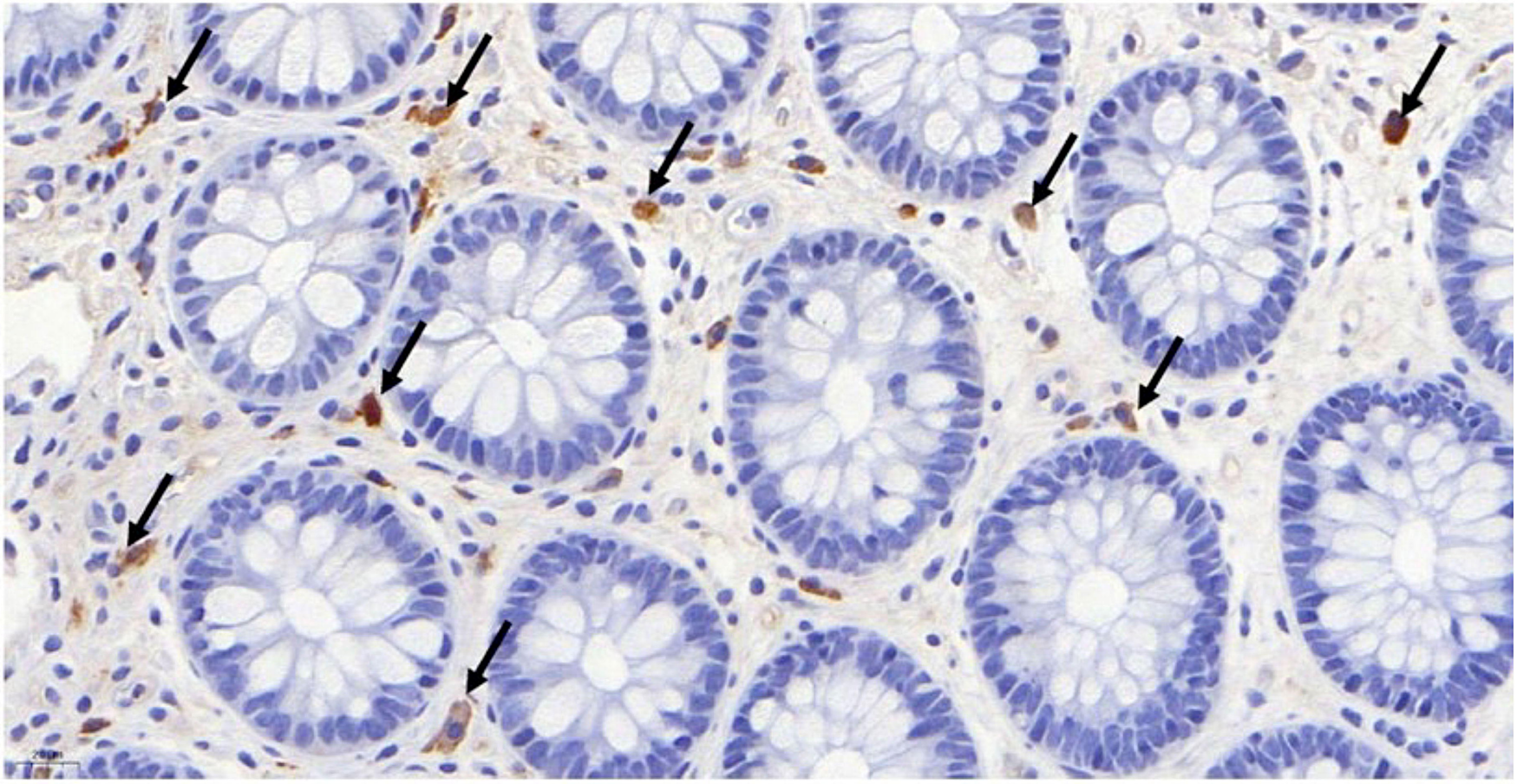
Legends for figures:

Figure 1: Histopathological example of IgA staining: Immunohistological staining of IgA + plasma cells – positive cells (arrows) were detected in the lamina propria with close association to the epithelial line

Figure 2: Cumulative treatment related mortality and IgA + plasma cells: 23 of 62 pts with low plasma cells died from TRM in contrast to only 11 of 61 pts with high IgA^+ plasma cells. Differences were significant (log rank p 0.015) A total of 115 pts and first biopsies were analyzed, time from biopsy is given.

Figure 3: Cumulative relapse related mortality is significantly increased in pts with high plasma cells (log rank p 0.03). Only 6/63 pts with low IgA^+ plasma cells died from relapse but 22/62 pts with high IgA^+ plasma cells. A total of 115 pts and first biopsies were analyzed

Figure 4: Competing risk analysis for TRM and RRM. Effects on RRM and TRM are independent (p for TRM 0.001, p for RRM 0.002)



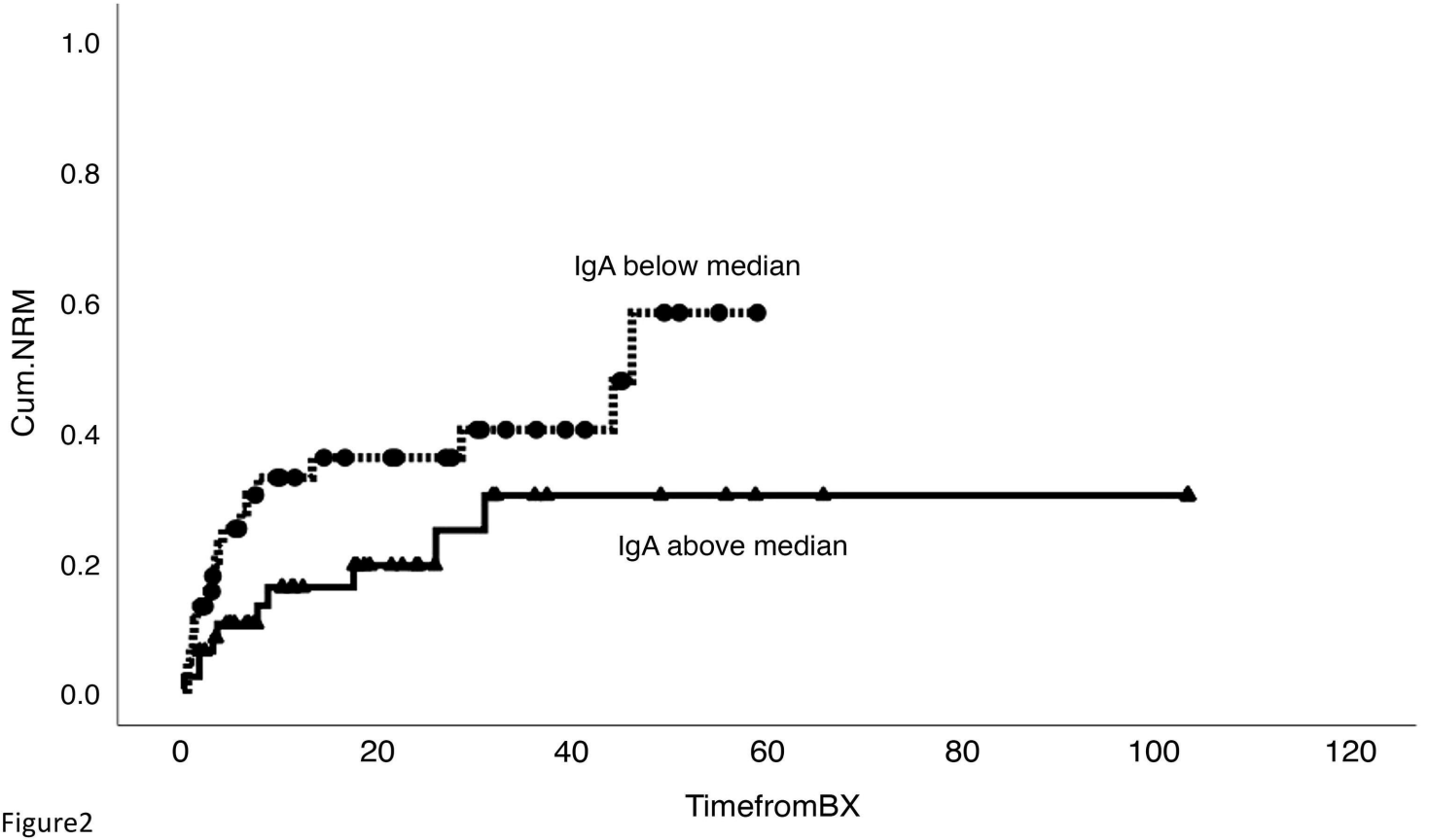

1.0
0.8
0.6
0.4
0.2
0.0
Cum.NRM
IgA below median
IgA above median
0
20
40
60
80
100
120
TimefromBX

Figure2

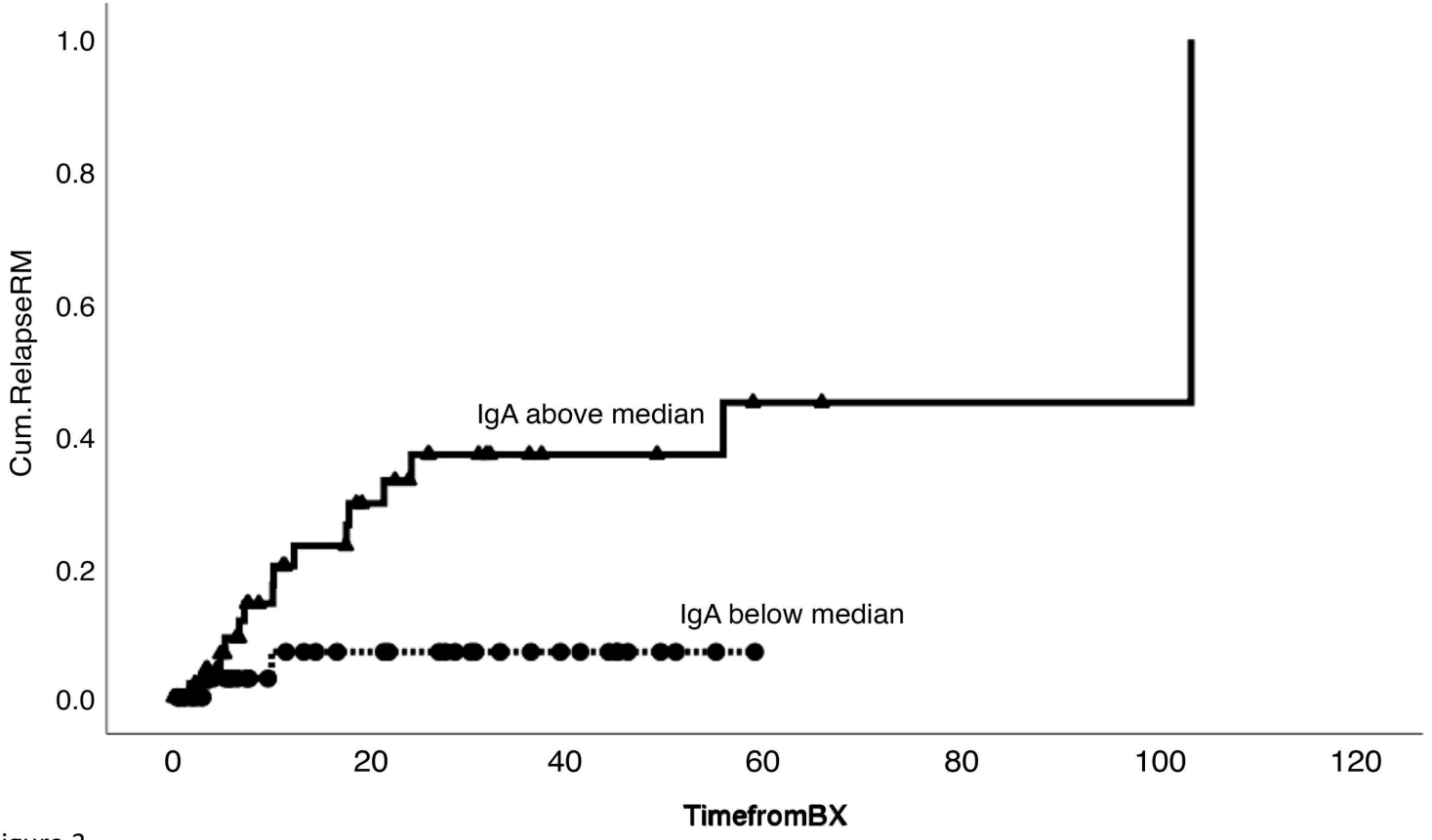
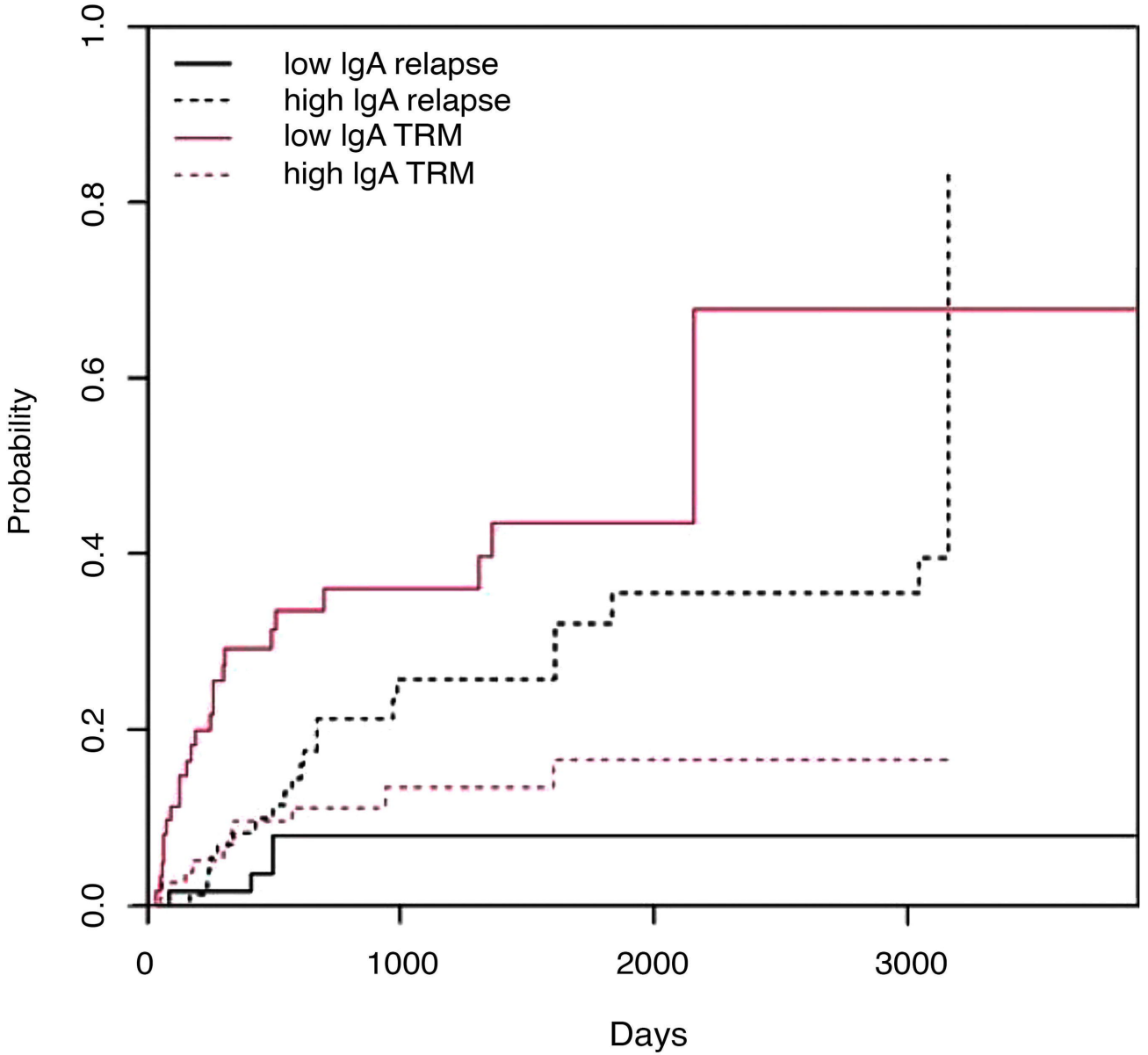

1.0
0.8
0.6
0.4
0.2
0.0
Cum.RelapseRM
IgA above median
IgA below median
0
20
40
60
80
100
120
TimefromBX

Figure 3

Figure4


low IgA relapse
high IgA relapse
low IgA TRM
high IgA TRM
Probability
0.0
0.2
0.4
0.6
0.8
1.0
0
1000
2000
3000
Days

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 ofsecondary 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 mm^2 per sample was determined and the cell concentration was calculated as the amount of IgA^+ plasma cells per mm^2. 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/mm^2 (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)

Supplemenal 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)