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Glycosylation of IgG B cell receptor (IgG BCR) in multiple myeloma: relationship between sialylation and the signal activity of IgG BCR

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Abstract Little is known about the glycosylation of the isotype switched B cell receptor (BCR) in multiple myeloma, and the way it might affect receptor function. In this work IgG BCRs isolated from the individual lysates of peripheral blood lymphocytes (PBL) of 32 patients with IgG multiple myeloma and healthy controls were investigated for the expression of sialic acid (SA), galactose (Gal) and *N*-acetylglucosamine (GlcNAc), the sugars known to specify the glycoforms of human serum IgG. The degree of glycosylation and signaling status of all 32 isolated myeloma IgG BCRs were correlated and compared with the glycosylation of the IgG paraproteins isolated from sera of the same patients. It was shown that BCR IgG in myeloma is more heavily sialylated when compared with normal controls, that the increased sialylation of IgG BCR is associated with higher levels of tyrosine phosphorylation (signaling activity) of the IgG BCR supramolecular complex and that BCR IgG and serum IgG paraprotein from the same patient differed in all cases in the levels of terminal sugar expression. The results suggest that the development of the malignant clone in MM from post-switch B cells expressing IgG BCR at their surfaces to plasma cells secreting IgG paraprotein may be followed by permanent glycosylation changes in the IgG molecules.

Keywords Multiple myeloma · IgG BCR · Signaling activity · Glycosylation

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Introduction

The B-cell antigen receptor (BCR) is a molecular complex composed of a membrane immunoglobulin of different isotype (class, subclass) associated with Igα (CD79a) and Igβ (CD 79b) signal transducing subunits (co-receptors). It binds antigen and triggers the signaling events determining the survival, progression, expansion and activation of the B cell [29, 30]. It also functions to endocytose bound antigen for subsequent intracellular processing and presentation with class II molecule [42]. Abnormalities in the BCR have often been associated with certain hematological malignancies in which structural or folding defects as well as functional deficiency of some components of this receptor have been observed [27]. The BCR constituents are glycoproteins and differences in glycosylation in any of them may contribute to the abnormal behavior of the whole receptor in a particular disease. Recently, Vuillier *et al.* [41] reported defects in glycosylation and folding in the μ and CD79a (Igα) chains associated with the lower levels of surface IgM expression in chronic lymphocytic leukemia (CLL) B cells, while Zhu *et al.* [46], Zabalegui *et al.* [45] and Radcliffe *et al.* [35] found a high incidence of potential glycosylation sites in the antigen binding sites of the BCR in follicular lymphoma (FL). A potential role for the BCR oligosaccharides in tumorigenesis, disease activity and progression has been suggested [25, 35].

Multiple myeloma (MM) is an incurable B-cell proliferative disorder characterized by a monoclonal expansion of malignant plasma cells (PC) and monoclonal immunoglobulin (paraprotein) in the serum of patients. Owing to the predominance of monoclonal IgG and IgA immunoglobulin classes, it has been hypothesized that MM derives from a proliferative subset of an isotype switched B cell clone (memory B cells or plasma blasts) that have passed through the phase of class switch recombination and somatic mutations [1, 34] and express BCR with IgG or IgA at their surface. Disease is often followed by abnormalities of cellular and molecular glycosylation. Thus, alterations in enzyme activities associated with glycosylation processes have been observed in plasma cells and peripheral mononuclear cells of myeloma patients [3, 4, 31]. IgG paraproteins were found to have abnormal oligosaccharide structures [12, 13, 31, 40] which, additionally, were localized at unusual sites within the molecules [23]. Moreover, analysis of IgG paraproteins showed that each has an essentially unique glycosylation profile which is assumed to reflect the unique properties of the clones producing them [11, 18]. These findings are indicative of the changes in glycosylation that could take place in the BCR complex expressed at the surface of B cells in myeloma. However, nothing is known about the glycosylation of BCR proteins in myeloma nor the way it might affect receptor function. We have recently shown for the first time that IgG BCR isolated from individual lysates of peripheral blood lymphocytes (PBL) from 32 newly diagnosed, untreated myeloma patients with monoclonal IgG (IgG paraprotein) in their serum (IgG myeloma) is in an activating state, with changes in the signaling status, when compared with healthy controls [17]. In this work we investigated the glycosylation profile of all these IgG BCRs, as defined by sialic acid (SA), galactose (Gal) and *N*-acetylglucosamine (GlcNAc) expression, and assayed whether there is a relationship between the expression of these sugars and the degree of IgG BCR signaling activity. SA, Gal and GlcNAc appear successively at the terminals of the outer arms of N-linked oligosaccharide chains in human serum IgG, and may be combined in different ways, thus specifying the glycoforms of IgG [19]. We also analysed each individual purified IgG paraprotein for the expression of these three sugars, and compared the results with those obtained for the glycosylation of BCR IgG of the same patient. The main findings are that BCR IgG in myeloma is more sialylated than in normal controls, that increased sialylation of myeloma IgG BCR correlates with the higher levels of tyrosine phosphorylation (signaling activity) of the IgG BCR complex, and that there are differences in the expression of terminal sugars between the BCR IgG and serum IgG paraprotein of the same patient. These results suggest that the development of the malignant clone in MM from post-switch B cells expressing IgG BCR at their surfaces to plasma cells secreting IgG paraprotein may be followed by permanent changes in the glycosylation of the IgG molecules.

Material and methods

Patients

A group of 32 unselected patients with newly diagnosed, untreated multiple myeloma (MM) was studied, after informed consent and with the approval of the local Research Ethics Committee. They had IgG paraprotein in their serum and monoclonal light chains in urine and >10% bone marrow plasma cells, together with clinical findings consistent with the diagnosis. According to the Salmon–Durie staging system 3 patients were in clinical stage IA, 8 in stage IIA, 12 in stage IIIA and 9 in stage IIIB. Among the 32 patients, 26 had IgG1, 3 IgG2, 2 IgG3 and 1 IgG4 paraprotein. Out of 26 IgG1 paraproteins, 22 carried the G1m(f) and 4 the G1m(az) allotype. IgG2 paraproteins carried the G2m(n), IgG3 the G3m(b1) and IgG4 the G4m (4b) allotype.

Cell isolation and preparation of cell lysates

Fresh mononuclear cells from peripheral blood (PBMC) of both the myeloma patients and healthy subjects were isolated by density gradient centrifugation using Lymphoprep separation medium (ICN Pharmaceuticals). Cells from the interface were recovered, washed three times and resuspended at a density of 5×10^6 cells/ml in RPMI 1640 culture medium (Sigma) supplemented with 10% FCS (Sigma). Cells were then incubated for 1 h at 37°C to deplete plasma antibodies, which might have been passively adsorbed onto the cell membranes [5]. After washing three times in RPMI medium without FCS, and once in PBS, 5×10^6 cells were lysed for 30 min at 4°C in 1 ml Nonidet P-40 (NP-40) lysis buffer (1% NP-40, 50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM Na_3VO_4, 10 mM NaF, 2 mM EDTA, 1 mM PMSF, 10 mM ε-ACA, 2 μg/ml aprotinin, 50 μg/ml leupeptin and 1 μM pepstatin A). Nonidet P-40 was used for cell lysis, because this detergent preserves the multicomponent structure of IgG BCR, keeping its constituents tightly linked [22]. After centrifugation at 10,000×*g* at 4°C, any insoluble debris was removed and the detergent-soluble cell lysates were used in the subsequent procedures. Protein concentration in the lysates was determined by the Bradford assay [2].

Isolation of IgG BCR

Whole lysates were first checked for the presence of IgG BCR. After SDS-PAGE under nonreducing conditions and immunoblotting with monoclonal anti-γ, anti CD79a and

anti CD79b antibodies, a fraction of 195–220 kDa was identified as reactive with these reagents. IgG BCR was then isolated as briefly described [7]. Cleared lysates containing 1 mg proteins (in a volume of 1 ml) were subjected to precipitation by adsorption to 100 μl of protein G-Sepharose beads (Amersham Biotech). This sorbent reacts with the sole IgG isotype irrespective of the IgG subclass. The lysates and beads were incubated at 4°C for 3 h, with continuous shaking. The protein complexes were collected by centrifugation and washed four times in NP-40 lysis buffer. Bound proteins were then released by boiling the samples in 250 μl of reducing SDS sample buffer. Normal IgG BCRs were isolated from NP-40 cell lysates obtained from ten healthy subjects included as controls after they had given informed consent. Immunoblot analysis of supernatants of protein G-treated lysates showed traces of unbound IgG in only four lysate supernatants, suggesting that the vast majority of IgG BCRs present in the lysates were isolated by the method used.

Molecular mass determination

Molecular size markers (Amersham Biotech) were subjected to 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The retardation factor (R_f) was calculated by plotting the electrophoretic mobility of each marker against its molecular mass and a standard curve was constructed.

Identification of BCR constituents

Samples of 10 μg isolated BCR (BCR isolates) were subjected to 10% SDS-PAGE. Total proteins were visualized by a silver staining procedure. The BCR constituents were identified after transfer to nitrocellulose membrane (Amersham Biotech). The membrane was blocked with 3% BSA in TBS and incubated at room temperature for 2 h with the following primary antibodies: mouse monoclonal anti-human γ chain (MH16-1ME, CBL, The Netherlands), goat polyclonal anti-CD79a (sc-8502) and goat polyclonal anti-CD79b (sc-8504) (Santa Cruz Biotech, CA), for IgG, Igα co-receptor and Igβ co-receptor identification respectively. Horseradish peroxidase-conjugated anti-mouse or anti-goat IgG antibody (Amersham Biotech) was used for immunodetection and, after incubation at room temperature for 1 h, BCR constituents were visualized with chloro-1-naphthol as the peroxidase substrate.

Analysis of protein tyrosine phosphorylation

IgG BCR isolates (10 μg) were loaded onto 10% SDS polyacrylamide gel for electrophoresis. The size-separated proteins were transferred to nitrocellulose membrane (Amersham Biotech). To detect tyrosine-phosphorylated substrates, membranes were incubated with mouse monoclonal anti-phosphotyrosine antibody (PT-66, Sigma) at room temperature for 2 h, followed by horseradish peroxidase-conjugated goat anti-mouse Ig secondary antibody (Amersham Biotech) for 1 h at room temperature, using 4-chloro-1-naphthol as the peroxidase substrate. In order to verify the identity of the phosphorylated proteins of interest (co-receptors), blots were stripped of antibodies by incubation in stripping buffer (100 mM 2-ME, 2% SDS, 62.5 mM Tris–HCl, pH 6.7) at 56°C for 30 min, washed extensively, reprobed with specific antibodies and then developed using the enhanced chemiluminescence reagent system (Amersham Biotech) according to the manufacturer's instructions.

Isolation of monoclonal serum IgG

IgG paraproteins were isolated from individual patients sera by ion exchange chromatography on Q Sepharose (Amersham Biosciences, Buckinghamshire, UK), in combination with affinity purification on protein G-Sepharose [38]. IgG paraproteins were separated from fresh sera, and the samples were stored at −70°C until use. IgG subclasses of the isolated paraproteins were defined by Western blot [36], using mouse monoclonal antibodies specific for γ1, γ2, γ3 and γ4 heavy chains (Nordic, The Netherlands). Allotypic Gm markers of the isolated IgG paraproteins were determined by inhibition-ELISA [32].

Glycosylation analyses

The localization of sialic acid (SA), galactose (Gal) and *N*-acetylglucosamine (GlcNAc) within the isolated IgG BCR was determined by lectin immunoblot assay [39]. This involved a combined procedure of SDS-PAGE under reduction conditions and lectin binding to the heavy and light chains of BCR IgG and co-receptors, separated after reducting. Biotinylated lectins (Vector, Burlingame, CA) from *Sambucus nigra* (SNA), *Ricinus communis* (RCA-I) and *Griffonia (Bandeiraea) simplicifolia* (BS-II) were used to detect specifically α2-6 linked SA, β1-4 linked Gal and β1-2 linked GlcNAc sugar residues, respectively. The same analyses were performed with IgG paraproteins isolated from MM patients' sera. Sugar expression was quantitatively estimated by measuring the peak areas obtained after densitometric scanning of blots using IMAGEQUANT software (San Diego, CA) and after lectin ratio determinations.

Statistical analyses

Software Prism Pad, version 3.0 (San Diego, CA) was used for statistical analyses. Data were expressed as

means±standard deviations. Values of $p \leq 0.05$ were considered significant.

Results

IgG BCR composition and phosphorylation status in MM patients

We have previously confirmed [17] the presence of IgG BCR and the completeness of its structure in all myeloma and control IgG BCR isolates, analysed in this work (Fig. 1).

Besides IgG BCR constituents, protein G-Sepharose precipitated several other proteins of different molecular size, indicating a physical link between the IgG BCR and additional molecules. APT immunoblotting showed tyrosine phosphorylation of molecules co-precipitated with IgG BCR (Fig. 2). In normal IgG BCR isolates only weak APT reactivity of p56 and p61 was detected. None of the co-receptor fractions was seen as APT reactive. This phosphorylation pattern resembles that typical for resting B-cells. Myeloma IgG BCR isolates contained APT reactive Igα or Igβ co-receptor variants in 93.75% of cases, indicating an activated state for the IgG receptor in circulating myeloma B cells. Other proteins co-precipitated with myeloma IgG BCR were highly phosphorylated and, together with the co-receptors, led to total phosphorylation levels between 1.1- and 30-fold (7-fold on average) higher in myeloma IgG BCR isolates than in the control IgG BCR isolate.

Glycosylation of IgG BCR in MM patients

The expression of Gal, GlcNAc and SA All IgG BCR isolate samples were assayed by SDS-PAGE combined with lectinoblotting, using three lectins. Both normal and all myeloma IgG BCR isolate samples reacted with biotinylated RCA-I and SNA lectins. Reactivity with GS-II was observed in 23 myeloma IgG BCR samples, only. This result indicates that terminal sugars characteristic for serum IgG oligosaccharides are present in the IgG receptor complex as well. In all samples, a fraction of 58–60 kDa (shown above to represent the heavy chains of BCR IgG) was lectin reactive. RCA-I and GS-II reacted with the light chains in three, and SNA in nine samples too. In six BCR samples a fraction of 31 kDa corresponding to Igα co-receptor was SNA-reactive, while in one sample this fraction was also RCA-I reactive (Fig. 3).

The content of Gal, GlcNAc and SA These three sugars appear successively in the oligosaccharide chains of normal serum IgG, and the results for their reactivity with lectins are usually expressed as a ratio, thus permitting the relative amount of a particular residue to be determined. This way of expressing the results corrects for any variation in the amount of BCR IgG applied in the SDS-PAGE, or any increase in the number of oligosaccharide moieties. GS-II/RCA-I and RCA-I/SNA ratios were calculated for Gal and SA content in the BCR IgG respectively, after densitometric scanning of lectin blots. The results showed a higher GS-II/RCA-I and lower RCA-I/SNA ratio for myeloma BCR IgG than for normal BCR IgG, suggesting that BCR IgG in myeloma has a lower content of Gal and a higher content of SA, when compared with normal BCR IgG (Fig. 4). A total of 68.7% myeloma BCR IgG samples were hypogalactosylated, while 78% were hypersialylated. This result suggests that an increase in sialylation might be the main glycosylation abnormality of myeloma BCR IgG.

The relationship between IgG BCR tyrosine phosphorylation and SA content of BCR IgG

A regression analysis (Fig. 5) was conducted to establish the relationship between the degree of IgG BCR tyrosine phosphorylation (expressed as fold change relative to the control) and the sialylation of IgG BCR (expressed as a RCA-I/SNA ratio). The 32 individual values of these two variables were correlated. A statistically significant trend towards a decrease of the RCA-I/SNA ratio with the increase of total phosphorylation level was observed (Fig. 5a). A similar trend was obtained with the co-receptors' phosphorylation only (Fig. 5b). This suggests that the increase of sialylation and the activation state of IgG BCR could be associated in myeloma. No significant correlation was found between the subclass or allotype of the sialylated IgG in IgG BCR isolates and the degree of IgG BCR phosphorylation.

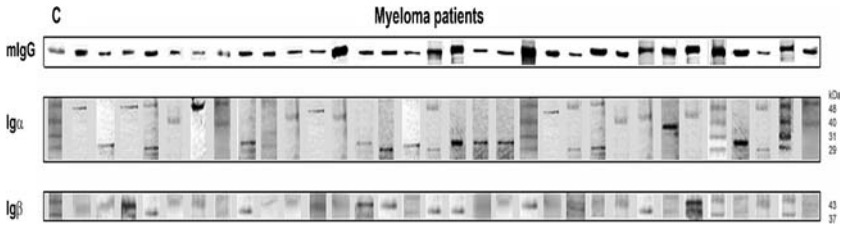

Fig. 1 IgG BCR constituents as identified by monoclonal anti-γ (*mIgG*), anti-CD79a (*Igα*) and anti-CD79b (*Igβ*) monoclonal antibodies; *C* control

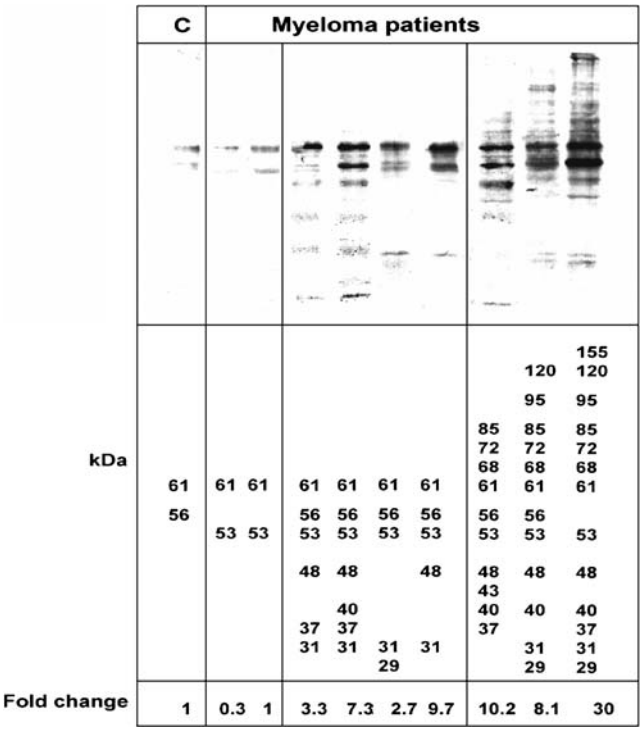

Fig. 2 Protein phosphorylation in control (*C*) and myeloma IgG BCR isolates. The data are representative of the three different phosphorylation patterns of myeloma IgG BCR observed [17]

Comparison of glycosylation profiles of IgG BCRs and IgG paraproteins in myeloma patients

Thirty-two paraproteins isolated from myeloma patients' sera were analyzed for Gal, GlcNAc and SA content by SDS-PAGE followed by lectinoblotting, using biotinylated RCA-I, GS-II and SNA. Each lectin reacted with heavy chains of all 32 paraproteins, and with the light chains of 16, 19 and 17 paraproteins respectively, reaching either higher or lower GS-II/RCA/I and RCA-I/SNA ratios in paraproteins, relative to the polyclonal control serum IgG (data not shown). Both ratios were shown to be lower in BCR IgG than in serum paraprotein of each individual patient, the RCA-I/SNA ratio being significantly lower (Fig. 6). This result is suggestive of possible continuous changes in the expression of outer arm sugars in IgG N-linked oligosaccharides within the clonal subset in myeloma.

Discussion

IgG BCR is expressed at the surface of isotypically-switched B cells. It controls clonal expansion of these cells and other signaling functions [24] that lead to production of plasma cells and IgG antibodies. In multiple myeloma, in which uncontrolled proliferation of B cells (mainly $IgG^{+}B$ cells) occurs, the clone appears to become independent of signals from the IgG BCR [17], and descendant plasma cells produce large amounts of monoclonal IgG (IgG paraprotein). Although various abnormal structures of IgG paraprotein oligosaccharides reflect immunological changes associated with monoclonal expansion of B lymphocytes in patients with IgG myeloma [18, 28], the glycosylation profiles of IgG BCRs have not been studied so far, either in myeloma patients or in healthy people. Also, the relationship between the glycosylation profiles of IgG BCR and any of its functions has remained uninvestigated.

The main finding we report here is higher GS-II/RCA-I and lower RCA-I/SNA ratios for myeloma BCR IgG than for normal BCR IgG. This suggests that BCR IgG in myeloma has a proportionally lower content of Gal, and a higher content of SA, when compared with normal BCR IgG. Typical N-linked glycans present at the conserved site in the Fc region of IgG are neutral, having Gal as the terminal sugar (preferentially on the man α(1→6) arm), and a low incidence of sialylation. Since galactosylation is essential for sialylation of IgG oligosaccharides, decreased galactosylation with simultaneous oversialylation may mean that SA was attached to an oligosaccharide structure

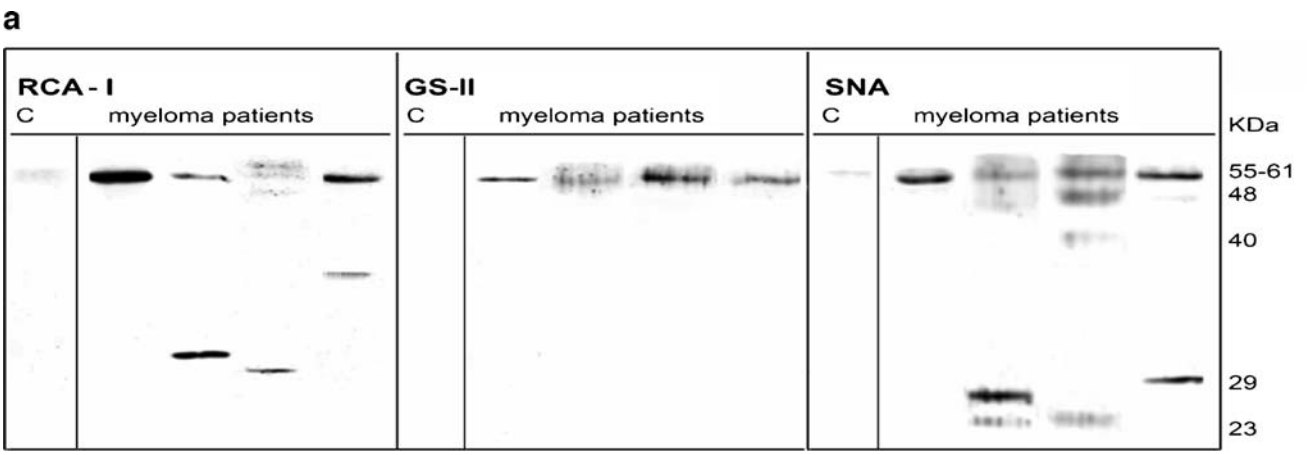

Fig. 3 RCA-I, GS-II and SNA reactive IgG BCR isolates: **a** representative lectinoblotting patterns; **b** three lectinoblotting densitograms for the same myeloma patient—representative samples of eight different patients; *n.r.* non-reactive; *C* control

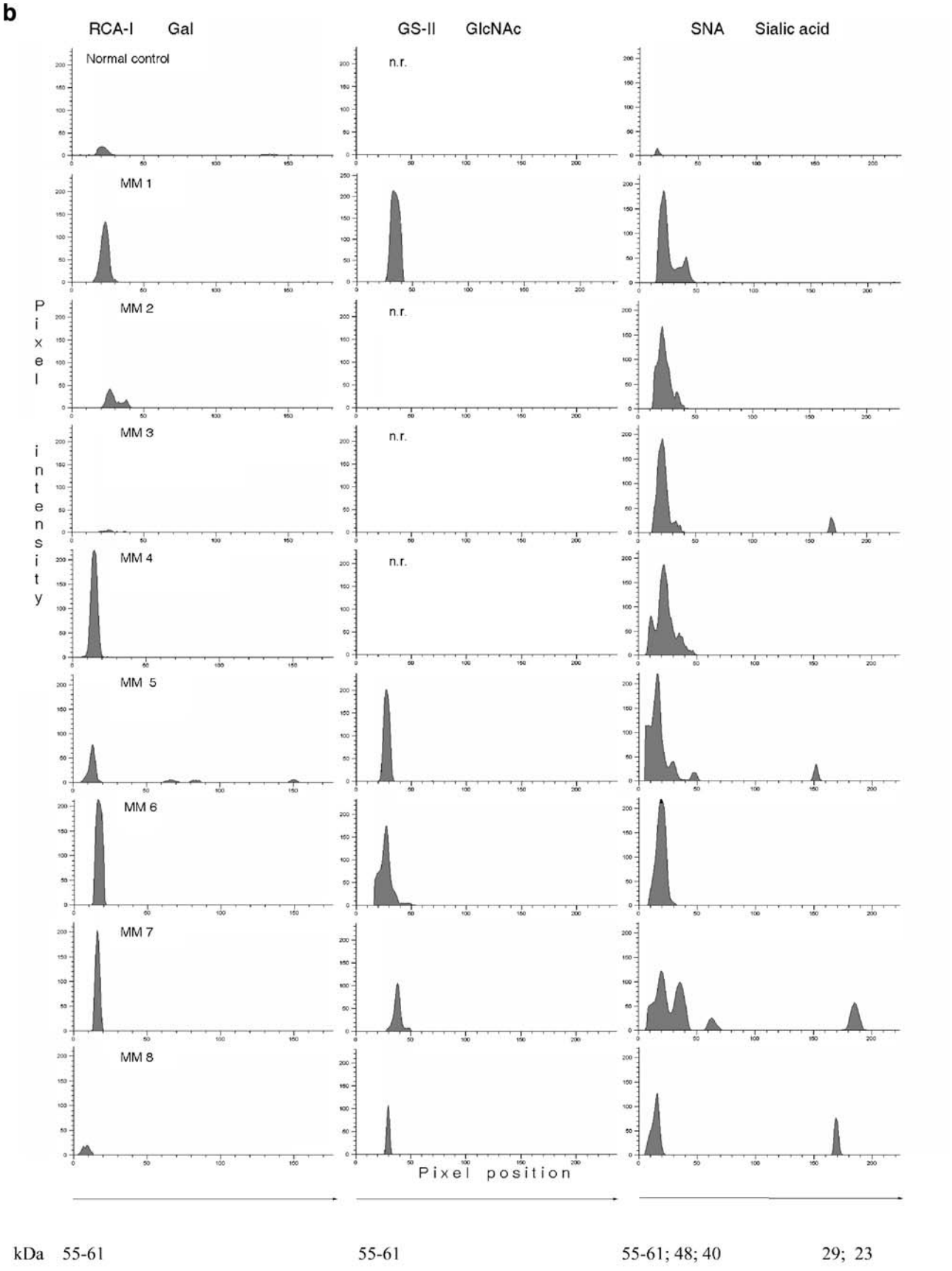

Fig. 3 (continued)

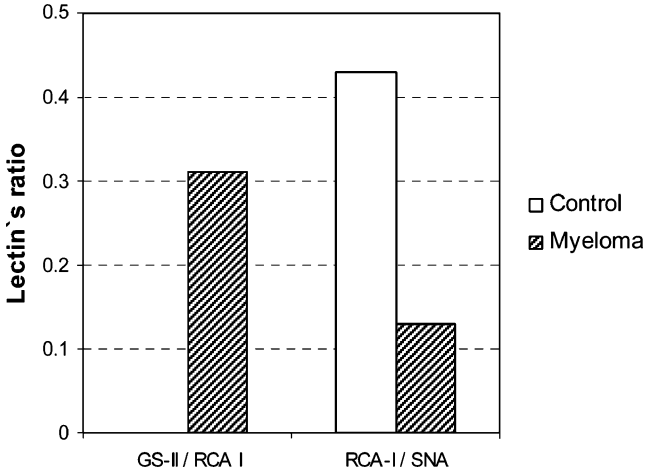

Fig. 4 The relative amounts of GlcNAc, Gal and SA in IgG BCR. The results are expressed as a lectin ratio

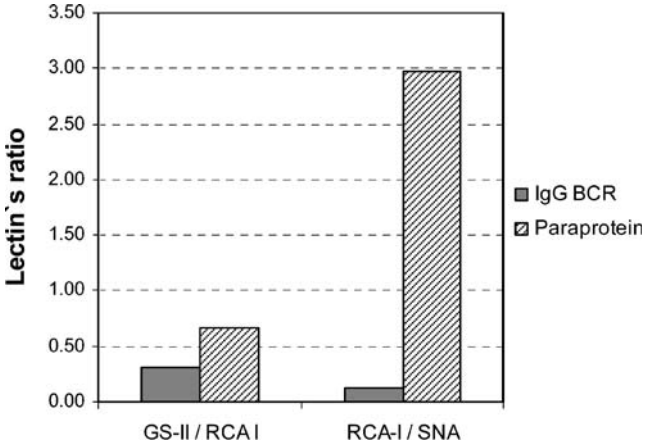

Fig. 6 The proportional amounts of GlcNAc, Gal and SA in BCR IgGs and IgG paraproteins in myeloma patients. The results are expressed as a lectin ratio

atypically present in some other part of the IgG. At present, we do not know the precise localization of SA in BCR IgG, particularly in its heavy chains. The sialylated light chains of some BCR IgG indicate that it could have been expressed on the Fab regions of the membrane IgG. Normally, IgG light chains are not glycosylated, but those isolated from human myeloma proteins have been found to contain N-linked oligosaccharides, which are presumed to be present on the variable regions [23, 26]. It is known that Fab regions may contain glycans of the highly sialylated complex-type or the high mannose type [23]. In human myeloma, Fab glycans occurred in about 25% of IgG paraproteins [9]. At the same time, increased sialylation of oligosaccharides on IgG paraproteins have been shown in myeloma relative to monoclonal gammopathy of undetermined significance (MGUS), which is a precursor state to MM [13]. However, novel sites for potential N-glycosylation in heavy chain V regions in myeloma displayed frequencies similar to those of normal cells, and in 8% of patients, only [46]. This is different from follicular lymphoma (FL), where such sites were found in almost 80% of patients [25, 46], and suggests that the over-expression of SA in myeloma BCR IgG is perhaps not due to the occurrence of new N-glycans in V regions of the BCR IgG heavy chains. There are no data on whether, under pathological conditions, highly conserved structural parts characteristic for surface immunoglobulin may be variably glycosylated. Nevertheless, membrane IgM does have glycosylated extracellular domains which, as such, contribute to its association with Igα and Igβ co-receptors [37].

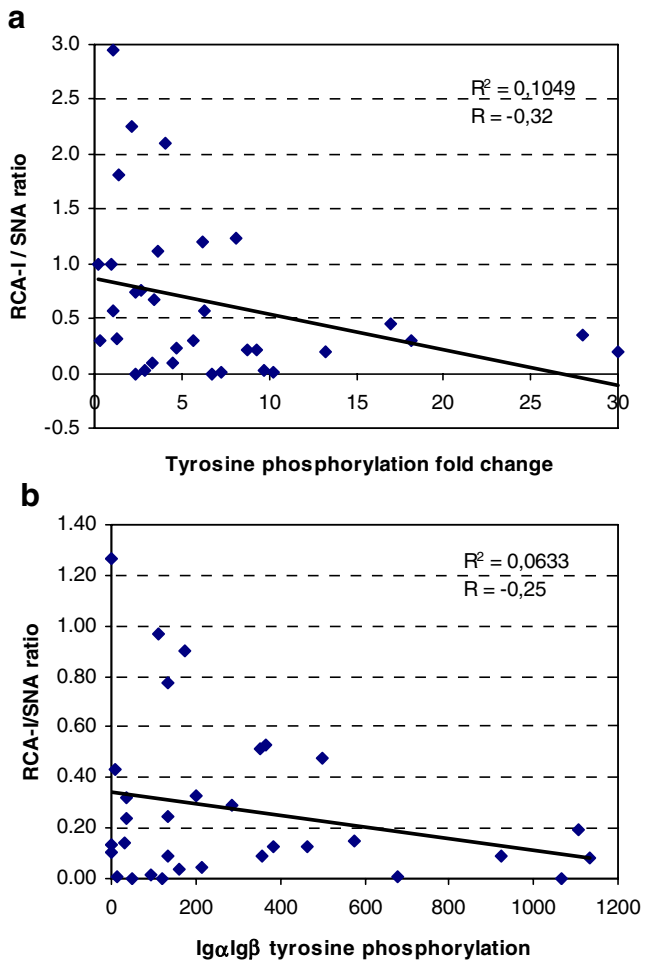

Fig. 5 Linear correlation between the degree of **a** total phosphorylation or **b** coreceptor phosphorylation (expressed as fold change relative to control) and the content of sialic acid (expressed as a RCA-I/SNA ratio)

The lower level of Gal and the excess of SA on membrane IgG from MM patients may be due to reduced activity of β1,4-galactosyltransferase (GalT) and hyperactive α2,6-sialyltransferase (ST) in B cells. Nishiura *et al.* [31] found hypogalactosylated IgG paraproteins and reduced GalT activity in bone marrow cells from patients with multiple myeloma, while Fleming *et al.* [13], Frithz *et al.* [14] and Cohen *et al.* [3] demonstrated oversialylation of IgG paraproteins, an increase in ST activity in serum and peripheral mononuclear cells in patients with myeloma, all suggesting

that the dysregulation of enzyme activities causing glycosylation abnormalities in immunoglobulins may perhaps reflect hyper-activation of B cells. Indeed, we found that the IgG BCR is in an activated state in myeloma [17] and that there is a correlation between sialylation of BCR IgG and IgG BCR activation state in this disease. A statistically significant trend towards a decrease of RCA-I/SNA ratio with the increase of total phosphorylation level was observed. A similar trend was obtained with the co-receptors' phosphorylation only, suggesting that the increase of sialylation and the activation state of IgG BCR could be associated in IgG myeloma. There are data showing that the signaling threshold for BCR may be adjusted by a mechanism in which surface Ig SA plays a role. In the resting phase of IgM^+ B cells, which is characterized by a low cellular expression of α2,6-ST [10], the endogenous sialic acid-reactive lectin, CD22, negatively regulates BCR signaling by binding to sialylated glycotopes of membrane IgM [6, 15, 20]. In the late phase of activation, when the B cells increase α2,6-ST expression, competition for binding to sialylated proteins other than surface IgM [6] draws CD22 away from surface Ig association and enhanced signaling is permitted [8]. The situation in myeloma appears to resemble that described above. The continuous immune-stimulation of isotype switched B cells, supposed to exist in myeloma [34], persistently directs IgG BCR towards proliferative signaling. That is probably why IgG BCR is in an activated state (our results) and the activity of ST in serum and peripheral mononuclear cells is increased [3, 4, 31]. Such conditions may prevent CD22, which is expressed on the plasma cell clonotypic precursors in myeloma [33], from being linked to the sialylated membrane IgG, thus permitting chronic enhancement of IgG BCR signaling and survival of malignant B cells in myeloma. Suggestive of this may be our finding that among phosphoproteins associated with the activated IgG BCR and coprecipitated with it during isolation there was no 140 kDa molecule, which is the predominant form of CD22. However, it has been shown in a mouse model that CD22-mediated signal inhibition, characteristic for IgM and IgD BCR, does not operate for IgG BCR. According to Wakabayashi *et al.* [43], it is prevented by the cytoplasmic tail of membrane IgG, thus causing IgG BCR to become more excitable to immune stimulation, while, according to Weisman *et al.* [44] and Horikawa *et al.* [16], the IgG tail enhances BCR-induced antibody responses by a mechanism that does not involve differences in the activity of CD22. However, the sialylation context was not considered in those studies.

We do not know at present whether the changes in Ig glycosylation begin to occur in the pre-switch cells, and whether they reverse or remain relatively stable as pre-switch B cells differentiate. Differences in glycosylation between the BCR IgG and IgG paraproteins, which we observed in myeloma, suggest that plasmacytes derived from B cells driven to class switching and terminal differentiation may continue to produce aberrantly glycosylated IgG. Interestingly, plasma cell IgG exhibited decreased galactosylation and sialylation, when compared with B cell IgG. This perhaps may be due to the fact that sialylation may affect the half-life of IgG in circulation. It is possible that the relatively low abundance of sialylated IgG in human serum (~15%) reflects a faster clearance of more highly sialylated molecules. It has been recently demonstrated [21] that the content of sialic acid in IgG varied during the evolution of the immune responses against antigens. At later stages of the response, during the process of affinity maturation, IgG antibodies switch to variants that lack sialic acid in their oligosaccharide structures. The clonal changes in myeloma observed to occur at the level of oligosaccharide compositions of monoclonal IgG may reflect the normal changes that take place during the development of IgG-secreting cells.

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