Influenza vaccination-induced B cell response in monoclonal gammopathy of undetermined significance (MGUS)
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Monoclonal paraprotein influences baseline B cell repertoire diversity and pertubates influenza vaccination-induced B cell response

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ABSTRACT

Introduction
Monoclonal gammopathy of undetermined significance (MGUS) arises from a monoclonal expansion of plasma cells in the bone marrow, secreting monoclonal (M) paraprotein. MGUS is associated with increased susceptibility to infections, which may reflect altered B-cell repertoire.

Methods
To investigate this, we examined the IgM, IgG and IgA B-cell repertoire diversity in MGUS at baseline and after influenza vaccination (n=16) in comparison to healthy controls (HCs) (n=16). The CDR3 region of the immunoglobulin heavy chain variable (IGHV) region gene was amplified and B-cell spectratypes analyzed by high-resolution electrophoresis. Spectratype Gaussian distribution, kurtosis and skewness were quantified to measure repertoire shifts.

Results
Both HC and MGUS baseline spectratypes show inter-individual variability that is more pronounced in the IGHG and IGHA repertoire. Overall, baseline B cell repertoire is more altered in MGUS, with oligoclonality observed in 50% (p=0.01). Post-vaccination, significant differences emerged in MGUS in relation to M-protein levels. High M-protein concentration is associated with a more oligoclonal IgG and IgA response at day 7 post-vaccination, and in contrast to HCs vaccination also induced significant perturbations in the MGUS IgM repertoire at day 7 (p=0.005).

Conclusion
Monoclonal expansion in MGUS has an effect on the baseline B cell repertoire and influences the recruited repertoire upon vaccination.
INTRODUCTION

Aging is associated with changes in the functioning of the immune system that is manifest as poor responses to infections and vaccination [1-3]. As antibody responses are the mainstay of protection against many infectious agents, recent investigations have focused on amelioration of the humoral responses in aging, including underlying B-cell repertoire shifts [4-6]. During aging, the emergence of a non-malignant condition like Monoclonal Gammapathy of Undetermined Significance (MGUS) in the elderly may further alter this susceptibility to infection or response to vaccination. MGUS is a condition that is generally identified surreptitiously, and arises from monoclonal expansion of plasma cells in the bone marrow, with potential to transform to full malignancy such as multiple myeloma [7, 8]. In MGUS, the expansion of plasma cells results in the secretion of elevated levels of monoclonal protein (M-protein), which can increase to reflect a further expansion [9, 10].

MGUS is associated with hypogammaglobulinaemia in 20-28% of cases [11-13] possibly reflecting an impact of the monoclonal clone on normal plasma cells in the BM niche. Accordingly, these changes in humoral capacity are likely to play a part in the increase in susceptibility to infection and decreased response to vaccination. A two-fold increased risk of bacteremia as well as a broad range of bacterial and viral infections has been described in MGUS patients compared to the general population [14]. These include pneumonia, influenza and herpes zoster infections [15, 16]. Furthermore, high monoclonal protein at diagnosis of MGUS has also been shown to be associated with higher risks of developing infections [15]. Besides the increased risk of infections, significantly depressed background antibody levels to a number of common infectious antigens have been reported, notably to staphylococcal, pneumococcal, varicella zoster and fungal antigens such as candida and aspergillus [16].

Responses to vaccination in MGUS remain poorly understood. Of infectious agents, influenza is a significant morbidity factor in aging and influenza vaccination is recommended in this high-risk group [17]. Influenza vaccination has an efficacy of 70% in healthy young adults [18], but the efficacy is decreased in the normal elderly population [19, 20]. In MGUS specifically, we have recently shown that the humoral immune response to influenza vaccination negatively correlates with the size of the M-protein [21]. MGUS patients with high M-protein levels elicited poor responses compared to age matched controls and this implies that high M-protein is a marker of underlying B cell dysregulation resulting in the poor immune response [21]. There is therefore a clear need to understand the nature of this immune dysregulation in MGUS patients, in order to develop strategies to counter their elevated susceptibility to infections and decreased response to vaccination.
B cell dysregulation impacts on the B cell repertoire and diversity can be mapped by analyzing the immunoglobulin variable region (IGV) gene repertoire [22]. The Complementary Determining Region 3 (CDR3) covers the junctional region where the 3 segments (IGHV-IGHD-IGHJ) are joined[23] forming the core antigen-binding site in an antibody molecule [22]. CDR3 diversity is therefore a critical measure of functional diversity of any B cell repertoire.

In a normal diverse B cell population a large number of clones derived from random V(D)J rearrangements result in a Gaussian distribution of CDR3 sizes. Where repertoire is perturbed, as with biases in repertoire usage or clonal expansions, the CDR3 distribution is altered and can be assayed by spectratyping [24, 25]. Spectratyping has been used to show that the B cell repertoire is compromised in the elderly. Aging is associated with oligoclonal expansions of cells and a collapse in the diversity of the B cell repertoire [4] that correlates with a general phenotype of frailty [26]. Vaccination has also been shown to induce changes in the B-cell repertoire. However, these changes are attenuated in aging and resolution of the response differs. Importantly, the IgM and IgA responses were impaired in the elderly and challenge resulted in smaller CDR3 sizes [4].

The onset of a monoclonal expansion in the elderly may further alter repertoire diversity, and thus far this has not been evaluated. To address this, we sought to delineate the baseline B cell repertoire in MGUS and humoral response to vaccination against influenza. We hypothesized that monoclonal plasma cell expansions in MGUS located in the BM may further alter the aging humoral response. To test this hypothesis, we analyzed the B cell repertoire using spectratype resolution of the CDR3 region of IGHV genes and modeled data to assess diversity in MGUS patients before and after influenza vaccination.

METHODS

Volunteers and sample collection

MGUS patients from the University Medical Centre Groningen hematology department and age-matched healthy controls were recruited during the 2010-2011 influenza vaccination season. The MGUS patients fulfilled the standard diagnostic criteria for MGUS: serum M protein levels <30g/L, clonal plasma cells in bone marrow <10% and no myeloma related dysfunction or other B-cell proliferative diseases. All participants gave written informed consent in accordance with the Declaration of Helsinki. The institutional medical ethics committee of University Medical Centre Groningen approved the study. All participants received influenza vaccination (Influvac 2010/2011, Solvay Pharmaceuticals, Netherlands) and blood samples were collected prior to the vaccination, as well as 7 days and 28 days post-vaccination.

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood by density gradient centrifugation of CPT Vacutainer tubes (BD). PBMCs were then
frozen in RPMI 1640 (Cambrex Bioscience, Verviers, Belgium) supplemented with 10% human pooled serum, 50 µg/mL gentamicin (Gibco, Paisley, UK) and 10% dimethylsulfoxide (Merck, Germany). PBMCs were stored in liquid nitrogen until further use.

**Nucleic acid extraction and cDNA synthesis**

Total DNA and RNA were extracted using the Qiagen Allprep DNA/RNA mini kit according to the manufacturers’ specifications. First strand cDNA was synthesized from RNA following standard protocols using Superscript-III (Invitrogen) in a reverse transcriptase system (Promega).

**Spectratypes**

cDNA was used for the amplification of the IGH gene CDR3 region using a PCR with primers specific for the constant regions Cα (GGAAGAAGCCCTGGACCAGGC), Cµ (CAGGAGACGAGGGGGAA) or Cγ (CACCGTCACCGGTTCGG) in combination with Fw3Fam (ACACGCTGTGTATTACTGT). The cDNA was amplified in 25 cycles of 98°C (45s), 45s annealing temperature of 61°C/55°C/55°C for alpha, gamma and mu respectively, 45s at 72°C and 1 final cycle of 5min at 72°C. This was done in a 25µl reaction mixture containing 20mM each of dNTP, 10µM of each primer (Fw3Fam plus one of the isotype specific primers), 0.2 Phusion DNA polymerase (NEB, Hitchin, UK) in a 5X reaction buffer containing 1.5mM MgCl₂. 5µl of each reaction sample was added to 1.5µl Tamra 350 size standard in formamide and run on the ABI 3730 xl capillary sequencer.

Multiple different methods and software tools for high CDR3 spectratyping analysis have been utilized; mainly on T cell repertoires [27-29]. However, in this study, raw spectratype data (fragment sizes) was analyzed and peak profiles visualized using Genescan software (Applied Biosystems) where the fluorescence intensity of each band was depicted as a peak. Data was then exported to Excel and frequency distributions of the CDR3 lengths were obtained. For further analysis, the Excel files were then imported into the R statistical programming environment (R Development Core Team, 2011) and the raw peak data was normalized so that the relative proportion of DNA fragments per CDR3 size was determined before spectratype curves were generated. A reference Gaussian distribution was generated using the mean and SD from each sample and B cell repertoire diversity was assessed by comparing the mean CDR3 size and correlating the spectratype to the reference Gaussian distribution (CGD). The divergence from the reference Gaussian distribution is therefore a direct measure of the corresponding repertoire diversity. To take account the shape of the spectratype distribution; the variability of the data, kurtosis and skewness were measured for each spectratype distribution. Kurtosis measures the distribution of the peaks, how much data is located at the tails as opposed to a distinct peak near the mean. Normally distributed data is given a value of zero while data that is
mainly centrally located near the mean and decline rapidly with a slim tail is given a
positive value (leptokurtosis, k > 0). A negative value is given to a distribution when
there is more data in the tails and the peak is flatter (platykurtosis, k < 0). Skewness
is a measure of symmetry of the distribution. For a symmetric distribution a value
of zero is given as the mean and median values coincide. A left/negatively skewed
distribution has a left tail that is long relative to the right tail and the mean of the
data is less than the median; values are less than zero. A right skewed distribution
has positive a value for skewness and the right tail is longer.

**Statistical analysis**

Statistical analysis was performed using Graphpad prism 5.0 (Graphpad Inc, USA). Mann-Whitney U test, Wilcoxon signed rank test and Chi squared test for
categorical data were used as appropriate. For correlations between different
measurements, Pearson’s correlation analysis was used. P-values < 0.05 were
considered statistically significant.

**RESULTS**

A total of 16 MGUS patients and 16 age-matched HCs were included in this
study (Table 1). Our MGUS cohort exhibited considerable heterogeneity in
terms of the type and size of M protein as well as duration of the condition. The
concentration of M-protein varied from unquantifiable levels to 24.8g/L. M-protein
was detected by immunofixation but was unquantifiable in 7 patients by serum
protein electrophoresis because the concentration was very low or the M-protein
migrated in the β fraction. A concentration of 0.01g/L was therefore assigned to
this subset of MGUS patients. The study demographics are shown in Table 1.

**Table 1. Baseline characteristics of MGUS patients and controls**

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls</th>
<th>MGUS</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>(n=16)</td>
<td>(n=16)</td>
<td></td>
</tr>
<tr>
<td>Age (years). median (Range)</td>
<td>63 (49-82)</td>
<td>64 (48-81)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>11/5</td>
<td>11/5</td>
<td>NS</td>
</tr>
<tr>
<td>Vaccination 2009/2010. N (%)</td>
<td>14 (87.5)</td>
<td>16 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of MGUS, years. Median (range)</td>
<td>NA</td>
<td>4(1-19.2)</td>
<td></td>
</tr>
<tr>
<td>Monoclonal protein size, g/L. median (range)</td>
<td>NA</td>
<td>6(0.01-24.8)</td>
<td></td>
</tr>
<tr>
<td>Monoclonal protein isotype. N (%)</td>
<td>IgG</td>
<td>NA</td>
<td>8 (50)</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>NA</td>
<td>4 (25)</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>NA</td>
<td>4 (25)</td>
</tr>
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</table>

NS, not significant; NA, not applicable.
Baseline analysis of the IgM, IgG and IgA repertoire shows more variability in MGUS

Spectratyping profiles can map polyclonality in a normal healthy immune humoral response arising from a homeostatic B-cell repertoire and accurately identify deviant repertoires that are altered by specific conditions. There are various alterations in the spectratype profiles that can be seen in a deviant repertoire. These alterations affect the shape and distribution of the spectratypes and can be modeled by CGD, kurtosis and skewness. Fig 1A shows representative spectratypes and the metrics measured from 4 healthy controls at day 0 and a comparative analysis of samples from 4 MGUS patients is shown in Fig 1B. In healthy controls, a polyclonal distribution of the CDR3 spectratypes was observed, although inter-individual variability occurred, as shown by the variations in CGD, kurtosis and skewness. The B cell repertoire was variable in MGUS with some showing polyclonal distribution of the CDR3 spectratypes similar to the healthy controls. Nonetheless, loss in diversity indicated by a few dominant expansions in the CDR3 sizes was observed in a subset of MGUS patients. These oligoclonal spectratypes (CGD<0.6) were observed in the MGUS baseline repertoire at higher frequency than in healthy controls. 50% of the MGUS patients had oligoclonal spectratype profiles for at least one of the three IGHV immunoglobulin gene repertoire in comparison to only one (6%) healthy control (data not shown, p=0.01).

A spectratype distribution was considered to be normal (approximately symmetric) if the skewness was between -0.5 and 0.5. However, a moderately skewed distribution had skewness between -1 and -0.5 or between 0.5 and 1 while a highly skewed distribution had skewness values less than -1 or greater than 1 [30]. Based on this interpretation, 3 MGUS IGHM spectratypes showed moderate skewness compared to 2 HC samples while 5 MGUS IGHA spectratypes were moderately skewed in comparison to 3 in HC. IGHG spectratypes showed increased skewness in MGUS, with 3 MGUS IGHG samples moderately skewed and 2 were highly skewed in comparison to none in HC, in which all spectratypes were approximately symmetric. Inter-individual variability in the HCs B cell repertoire was more pronounced in the IGHG (p=0.001) and IGHA (p=0.036) spectratypes than in IGHM. Of the healthy controls, 69% had IGHM CGD values of 0.9 or higher, indicating repertoire complexity as depicted by the large number of different sized CDR3 (Table 2).

We subsequently investigated whether age was associated with a less diverse repertoire in HC and MGUS. No age-related changes were observed in the IGHM, IGHG or IGHA spectratypes of the healthy controls. However, age was associated with a decrease in MGUS baseline IGHG CGD index, regardless of the monoclonal protein isotype, with a Pearson’s r of -0.55 (p=0.04) (Fig 1C).
Figure 1. IGHM, IGHG and IGH A spectratypes show inter-individual variability. (A) Spectratypes from 4 example MGUS patients at day 0 show inter-individual variability that is more pronounced in IgG and IgA. The x-axis represents the CDR3 sizes in base pair and the y-axis is the frequency of occurrence of the CDR3 sizes. The table shows how the spectratypes differ between individuals as measured by the mean CDR3 size, how close the individual spectratypes match a Gaussian distribution (CGD) and the difference in shape by kurtosis (k<0: platykurtic, k=0: normal, k>0: leptokurtic) and skewness (left-skewed distribution>0, right-skewed<0). (B) Spectratypes from 4 representative HCs show the CDR3 sizes and their distributions. Some spectratypes with a prominent (oligoclonal) CDR3 size have a high peak signal that results in lower match to CGD such as in donor 140 (IgM and IgG). (C) Age was associated with a significant decrease in IGHG CGD in MGUS patients.
Vaccination induces change in the MGUS B-cell repertoire that correlates with M-protein load

Following encounter with antigen, specific B cells expand and this leads to the change in baseline repertoire distribution (Fig 2A,B). There was a pronounced shift in the shapes of the spectratypes at day 7 that then return to the baseline shape at day 28. Some profiles in the MGUS patients indicated a diverse repertoire at day 0. However, in response to vaccination, there are marked differences in the spectratypes between patients (Fig 2B). Perturbations in the repertoire evidenced by oligoclonal CDR3 profiles and lower CGD were seen in some patients at day 7 that do not return to baseline values at day 28 but remain oligoclonal. In 3 MGUS patients, a perturbed repertoire with a dominant CDR3 length was seen at day 7 in response to vaccination. However, on day 28, the spectratypes remained oligoclonal but with a different pattern as a different sized dominant peak was seen (data not shown). Interestingly, distortions in some of the MGUS spectratype profiles that could be related to the monoclonal expansion of B cells in MGUS were observed in 3 of the patients. In these patients, the spectratype profiles had a major CDR3 length that is dominant in the repertoire, demonstrated as a peak expansion. The major CDR3 length dominated the profiles at all 3 time-points, before and after vaccination (Fig 2C). Of note, the dominant CDR3 length was seen in the isotype of the spectratype profile that matched the isotype of the M-protein from the MGUS patient, for example: the peak expansion was observed on IGHG spectratypes for an IgG MGUS patient.

Influenza vaccination resulted in change in CGD index of the spectratypes that were mostly in the IGHG and IGHA isotypes but not in IGHM in the healthy controls. MGUS patients show a trend towards having a lower baseline IGHG repertoire diversity (p=0.07) in comparison to healthy controls (Fig 2D). Nonetheless, a significant change in the CGD similar to that of healthy controls was observed for IGHG (p=0.05) and IGHA (p=0.03) spectratypes day 7 post-vaccination. In contrast to healthy controls, there is significant repertoire perturbation in the MGUS IGHM spectratypes at day 7 (p=0.005) with the CGD returning to baseline values at day 28. This resulted in significantly lower IGHM CGD values for MGUS at day 7 in comparison to healthy controls (p=0.006).

Table 2. Samples matching Gaussian distribution; CGD ≥ 0.9

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>MGUS</th>
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<tr>
<td>IGHM N (%)</td>
<td>11/16 (68.8)</td>
<td>9/16 (56)</td>
</tr>
<tr>
<td>IGHG N (%)</td>
<td>6/16 (37.5)</td>
<td>2/16 (12.5)*</td>
</tr>
<tr>
<td>IGHA N (%)</td>
<td>7/16 (43.8)</td>
<td>3/16 (18.8)*</td>
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</table>

* Significantly lower MGUS samples matching CGD than IGHM samples, p<0.05
To investigate whether influenza vaccination has an influence on the immunoglobulin IGHV gene repertoire selection we looked at the change in mean CDR3 sizes. No change in the CDR3 size was observed in healthy controls following vaccination. Perturbations occur at day 7 and the spectratypes returning to day 0 shapes at day 28. Representative spectratypes from 3 MGUS patients show that there are variations in the spectratype patterns following influenza vaccination. Spectratypes from 3 MGUS patients where monoclonal expansion of cells of a particular CDR3 size dominate the spectratypes before and after vaccination. MGUS donor number and spectratype isotypes are shown on the right y-axis. Vaccination induced change in repertoire is shown by the change in CGD following vaccination. Change in mean CDR3 length following vaccination. Monoclonal protein concentration negatively correlates with day 7 CGD values for IGHG and IGHA spectratypes. *p<0.05; ** p<0.01.

Figure 2. Vaccination results in changes in the B cell repertoire. (A) Spectratypes from a HC donor at day 0 and, day 7 and day 28 post-vaccination. Perturbations occur at day 7 and the spectratypes returning to day 0 shapes at day 28. (B) Representative spectratypes from 3 MGUS patients show that there are variations in the spectratype patterns following influenza vaccination. (C) Spectratypes from 3 MGUS patients where monoclonal expansion of cells of a particular CDR3 size dominate the spectratypes before and after vaccination. MGUS donor number and spectratype isotypes are shown on the right y-axis. (D) Vaccination induced change in repertoire is shown by the change in CGD following vaccination. (E) Change in mean CDR3 length following vaccination. (F) Monoclonal protein concentration negatively correlates with day 7 CGD values for IGHG and IGHA spectratypes. *p<0.05; ** p<0.01.
vaccination for both IgM and IgG. However, there was a significant increase in the mean CDR3 length at day 7 in the IgA repertoire (p=0.05) that then returned to smaller length at day 28 as seen at baseline. There is evidence of repertoire bias towards use of longer CDR3 length in the IgG and IgA repertoire but not in IgM for MGUS patients in response to vaccination (Fig 2E). The mean CDR3 length at day 7 in MGUS was significantly higher than in healthy controls (p=0.008) for IgG.

M-protein levels significantly correlated with CGD at day 7 in the IgG and IgA repertoire (Fig 2F). Higher M-protein concentration was associated with more oligoclonal response at day 7 in the MGUS IGHA repertoire, with CGD as low as 0.1, indicating expansions in a few distinct CDR3 sizes ($r^2 = -0.51$, $p=0.04$). This notion that a few CDR3 sizes expand in response to vaccination is supported by the observation that leptokurtosis was significantly correlated with M-protein at day 7 for the IGHA spectratypes ($r^2 = 0.66$, $p=0.005$). Additionally, CGD at day 7 was also negatively correlated with M-protein concentration for the IGHG spectratypes ($r^2 = -0.66$, $p=0.005$) but not for IGHM. Further stratification of the MGUS patients into 2 groups based on M-protein concentration showed that M-protein >10g/L (n=7) was associated with a significantly lower CGD at day 7 post-vaccination for both IGHA (p=0.0164) and IGHG (p=0.05) in comparison to MGUS with M-protein concentration <10g/L.

Furthermore, we investigated whether a less diverse repertoire could predict a worse response to vaccination. H1N1-specific IgG responses were established in the same MGUS cohort as for this study and were recently reported separately [21]. We therefore looked for correlation between the spectratypes metrics and H1N1 influenza-specific IgG responses. No correlations were observed for the healthy controls. However, lower CGD at day 7 in the IGHM repertoire was associated with lower H1N1 influenza-specific IgG responses at day 28 in MGUS ($r^2 = 0.55$, $p=0.02$). Those with higher serum IgG response showed less perturbation in the IGHM repertoire following vaccination (Fig 3).

![Figure 3. H1N1-specific IgG response at day 28 correlate with IGHM spectratypes at day 7.](image-url)
DISCUSSION

In this study we have investigated the B cell repertoire in MGUS by looking at CDR3 spectratypes at baseline and examined the effect of vaccination on the IGHM, IGHG and IGHA CDR3 spectratypes. Naïve B-cell repertoires show a characteristic Gaussian distribution of CDR3 length diversity reflecting polyclonality of the B cells. Deviation from Gaussian distribution is considered to be a sign of oligoclonal expansions. There were inter-individual variations in the spectratype metrics in all 3 isotypes that were more prominent in the IGHG and IGHA spectratypes for both healthy controls and MGUS patients. The IGHG and IGHA samples contain isotype switched B cell populations and reflect an individual’s prior antigen exposure so inter-individual variation was expected in these repertoires.

Oligoclonality of the baseline spectratypes indicating lack of repertoire complexity was observed in 50% MGUS patients. It is unclear at present what underlies the increased frequency of perturbations in the MGUS peripheral B cell repertoire. The host immune system is capable of responding to pre-malignant cells [31-33], and it is plausible that the immune response against specific antigens in MGUS [34] may also lead to an alteration in the baseline repertoire that will play a role in shaping the repertoire available to respond to vaccination. This chronic immune activation has a potential in suppressing the neoplastic growth [32] but may also result in a restricted (memory) B-cell repertoire with an exhausted ability to generate expansion responses to vaccination, possibly related to the decrease in baseline IGHG CGD in MGUS, with IGHG highly relevant to B-cell response to infectious agents. The relationship between the MGUS clone and its microenvironment is important for the maintenance of the clone in the bone marrow but could also induce immune dysfunction. The observation of dendritic cells suppression in MGUS may be related to increased immunosuppressive cytokines such as IL-6 [35] that interfere with dendritic cell maturation and antigen presenting capacity. Increased regulatory T cells in MGUS may also play a role in modulating the immune response [36]. Furthermore, we reported a decrease in the relative numbers of CD19+ B cells in this cohort of MGUS patients as well as a decrease in total serum IgG and IgA [21]. Hypogammaglobulinaemia is reported in ~25% of MGUS, and this may reflect the impact of the monoclonal plasma cells on pan-antibody production [13] as clonal plasma cells compete with diverse normal plasma cells for bone marrow niche space [37, 38]. When we linked B-cell repertoire diversity to age in a healthy elderly cohort and contrasted these with aged individuals with MGUS, no age related differences in repertoire diversity were observed in the healthy controls. However, increased age was associated with a less diverse IGHG repertoire in MGUS indicating that other than the aging-related-alterations in peripheral B cell compartment, there are also defects intrinsic to MGUS that may contribute to the repertoire alteration seen with age. The potential immunosuppression in MGUS could then make this
The majority of the HCs and all the MGUS patients were vaccinated the previous year with the same H1N1 influenza strain that was included in the TIV vaccine. Therefore it is likely that the switched isotypes contain influenza specific memory B cells that will rapidly proliferate and differentiate in response to the second vaccination generating plasma cells and a discernible rise in serum antibodies with IgG being the most relevant for protection [39, 40]. There is less perturbation in the healthy controls B cell IGHM repertoire at day 7 post-vaccination presumably because a recall response is elicited to influenza vaccination. Influenza specific antibody secreting cells (ASCs) are also detected in the peripheral blood and peak 1 week after vaccination that are predominantly IgG and IgA [39] consistent with the timing of the repertoire perturbations 7 days post-vaccination. As we used RNA for analyzing immunoglobulin sequences of total peripheral B cells, and ASCs are known to produce >100-fold more mRNA than resting B cells, the change in the spectratype shapes at day 7 may also reflect the greater proportion of ASC expansion more than mature B cells. In contrast to healthy controls, vaccination induces significant perturbations in MGUS IGHM repertoire at day 7 that returns to baseline pattern at day 28. Of note is that the IGHM spectratypes reflect a repertoire that largely consists of naïve B cells [41] therefore the change at day 7 suggests a perturbation of the antigen-inexperienced repertoire in MGUS. However, clonal expansions within the IgM memory population cannot be excluded in these MGUS patients. Moreover, the perturbations in the MGUS IGHM repertoire observed 7 days post-vaccination are shown to correlate to vaccine response with a perturbation in the repertoire being associated with a worse H1N1 influenza-specific IgG response.

A striking observation is that high monoclonal protein is associated with more oligoclonal response at day 7 in both the IGHG and IGHA spectratypes in MGUS. Multiple myeloma monoclonal protein reflects the monoclonal plasma cell burden and it is highly likely that the relationship is the same in MGUS in which high monoclonal protein is associated with higher risk of progression [12]. This implies that high monoclonal protein is a marker of increased underlying B cell dysfunction, limiting the pool of B cells available to respond to antigenic challenge. As a consequence, a limited B cell repertoire responds to the vaccine challenge in MGUS. The observation that some spectratypes remain perturbed at day 28, but with a different sized dominant peak, suggests that many different factors may play a role in shaping the responding repertoire. It may relate also to the slow resolution of infection or another kinetic aspect of the humoral immune response altered by MGUS cells. Whether the different sized dominant peaks observed are due to an independent effect such as a secondary infection subsequent to vaccination...
remains uncertain. Spectratype distributions with a major CDR3 length of the same size dominated the profiles at all 3 time-points before and after vaccination in 19% of MGUS patients, of the same isotype as secreted M protein secreted and are most likely to reflect circulating MGUS monoclonal cells. Circulating aberrant monoclonal plasma cells have previously been detected by immunofluorescence microscopy and flow cytometry in up to 20% of MGUS [15-17].

Our results are in contrast to Ademokun et al’s observation that pneumococcal vaccination resulted in expansion of clones with smaller CDR3 sizes in healthy controls [4]. MGUS spectratypes assessed in our study show that there is a preferential expansion of longer CDR3 in the IGHG and IGHA repertoire at day 7 post-vaccination. The significance of this finding is however unclear but the differences could be due to the differences in antigens and immune pathways of response as pneumococcal vaccination results in a TI-2 response and no memory while influenza vaccination results in a T-dependent response. It may also indicate differences in expression of IGHV and IGHJ genes. Long CDR3 lengths have been associated with self-reactive or polyreactive B-cells [42, 43]. Furthermore, antibodies with long CDR3 are shown to be enriched in pre-B cells and immature cells [44]; however CDR3 lengths are discerningly decreased as B cells progress through development. Memory B cells that have shorter, more hydrophilic CDR3 regions are usually more selected while naïve B cells generally have the longest CDR3 regions and less mutations [41, 43, 45]. Our results are consistent with these findings in that the IGHM repertoire consists of the longest CDR3s.

In summary, these results define the impact of MGUS cells on humoral immunity, and the impact of monoclonal protein burden on perturbations of the B cell repertoire induced by vaccination. We have shown that B cell repertoire diversity is altered in MGUS with oligoclonal expansions being seen in a subset of the patients. It remains to be investigated how MGUS results in the disturbance of B cell subsets composition and memory maintenance, and these studies are currently in progress in our laboratory.

REFERENCES


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