Immunoglobulin G Subclass Analysis in Psoriatic Arthritis

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ABSTRACT. ***Objective.*** The occurrence of monoclonal gammopathy of undetermined significance (MGUS) is common in chronic immune mediated disorders. This increased monoclonal antibody production could result from chronic stimulation of lymphocytes, with the immunoglobulin G (IgG) subtype accounting for the majority of cases in psoriatic arthritis (PsA). We aimed to identify IgG subclass profiles in patients with PsA and to determine association with specific disease characteristics.

Methods. Serum samples from 221 patients with PsA from a single cohort were analyzed for their serum IgG subclass levels. All patients fulfilled the ClASsification for Psoriatic ARthritis (CASPAR) criteria and were followed at 6-month to 12-month intervals according to a standard protocol. MGUS was defined as the occurrence of a discrete band in the gammaglobulin region on at least 2 separate serum protein electrophoresis tests performed 6 months apart. Patients with high abnormal IgG subclass levels were compared to patients with normal levels using descriptive tests.

Results. Elevations of IgG1-4 were common in PsA, with ~20%–49% of patients having elevations of each subclass, IgG2 being the most common subclass abnormality. However, no clinical-serological correlation was found in the group with abnormal IgG2 levels. Of the 38 patients with MGUS, elevations in IgG1 were most common. Patients with an abnormal IgG1 subclass level were more likely to have a discrete band in the gammaglobulin region, higher prevalence of MGUS, and abnormal erythrocyte sedimentation rate or C-reactive protein levels.

Conclusion. Determination of the IgG subclass concentration in PsA did not seem to add any significant value in identifying specific disease manifestations. However, this study provides insight into the pathological process leading to MGUS in PsA. (J Rheumatol First Release Oct 15 2014; doi:10.3899/ jrheum.131477)

Key Indexing Terms:
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Human immunoglobulin G (IgG) consists of 4 subclasses that differ in their heavy chain constant regions and that are named based on their abundance in the serum (IgG 1–4)[1,2]. These subclasses have differing abilities in microbial opsonization and activation of the complement pathway. They exhibit a restricted response to specific pathogens, in which antibody responses to protein antigens and viral antigens are composed predominantly of IgG1 and IgG3[3,4], whereas IgG2 is induced to capsular antigens[5]. Production of the different IgG subclasses is dictated by the cytokine milieu, with proinflammatory cytokines such as those implicated in Th1 and Th17 pathways, as well as in keratinocyte proliferation, including interleukin (IL)-2[6], IL-6[7], and interferon (IFN)-α[8] playing a pivotal role.

The occurrence of monoclonal gammopathy of undetermined significance (MGUS) is common in chronic inflammatory and immune-mediated disorders. Studies of patients with rheumatoid arthritis (RA) positive for anticyclic citrullinated peptide antibodies showed predominantly abnormal IgG1 levels as well as conspicuously elevated IgG4 levels, indicative of a Th2-biased environment[9]. Although psoriatic arthritis (PsA) is considered a seronegative T cell mediated disease, a variety of proinflammatory cytokines are produced that are also implicated in the humoral response. In a previous study[10], we found that the prevalence of MGUS in PsA was 9.7%, much higher than the overall prevalence in whites, which is estimated to be about 1.5% in those above the age of 50 years and 3% in those above the age of 70[11]. Moreover, MGUS in PsA was associated with

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longer disease duration, increased age, and higher measures of disease activity including erythrocyte sedimentation rate (ESR) levels, but not with damage or specific disease manifestations. This supports the notion that chronic stimulation of lymphocytes could lead to clonal transformation.

The majority of MGUS are IgG isotype, suggesting that their presence may arise from polyclonal activation of IgG-secreting B cells. However, to our knowledge, no study has investigated IgG immunoglobulin subclass levels in PsA and whether there is an association between specific patterns of disease, or the presence of MGUS, with any of the IgG subclasses.

The aims of our study therefore were to identify IgG subclass profiles in patients with PsA and to determine associations with specific disease characteristics such as axial or peripheral arthritis, enthesitis, uveitis, and inflammatory bowel disease (IBD), and the presence of MGUS.

MATERIALS AND METHODS

Serum samples from consecutive patients with PsA and a selected group of patients with PsA and MGUS followed at the University of Toronto PsA clinic were analyzed for IgG subclass using the MILLIPLEX Human Immunoglobulin Isotyping Magnetic Bead Panel (EMD Millipore). All patients fulfilled the ClASsification for Psoriatic ARthritis (CASPAR) criteria[12] and were followed at 6-month to 12-month intervals according to a standard protocol that collected data on patients' demographics, disease characteristics, and background therapy. MGUS was defined as the occurrence of a discrete band in the gammaglobulin region on at least 2 separate serum protein electrophoresis tests performed 6 months apart. Descriptive statistics included mean (SD) for continuous variables and frequency (percent) for categorical variables. Patients with a high abnormal IgG subclass level were compared to patients with normal levels using t tests, Fisher's exact test, and chi-square tests. Statistical analyses were done using SAS 9.3. Abnormal levels were defined as provided in the Certified Reference Material 470[13].

RESULTS

Serum samples were tested from 221 patients with PsA who were attending the PsA clinic. These included 183 consecutive patients who lacked MGUS, and 38 selected patients with PsA and known MGUS. The demographics and disease characteristics of the patients are depicted in Table 1.

Within the MGUS-negative group, the mean age was 54.7 years (12.5), with 62.8% male. Patients had a mean disease duration of 16.6 years (12.0). Of the study group, 2.2% had a history of IBD, 12.6% had anterior uveitis, and 18.6% had a history of preceding infection. Regarding treatment, 52.5% of the study group were treated with disease-modifying antirheumatic drugs, and 50.6% were taking biologics. Only 0.9% of the patients were seropositive for rheumatoid factor (RF).

The 38 patients with MGUS were older (61.3 yrs vs 54.7, p = 0.003), with a longer duration of psoriasis (33.1 yrs vs 26.0, p = 0.007) and PsA (23.1 yrs vs 16.6, p = 0.007). They had higher active (11.1 vs 5.4, p = 0.04) and damaged (16.3 vs 11.2, p = 0.05) joint counts, higher ESR (21.5 vs 12.1, p = 0.04), but no difference with respect to treatment.

The mean (% elevated) levels, in the 221 patients, of IgG subclasses IgG1, IgG2, IgG3, and IgG4 were 856 (33.5%), 635 (47.1%), 141 (21.7%), and 157 (20.3%) mg/l, respectively, with IgG2 being the most common subclass abnormality. Similar results were obtained when analyzing the data on the 202 consecutive patients (30.2%, 49%, 21.3%, and 20.3%, respectively). Comparison of patients with and without MGUS revealed a trend to higher levels of IgG1 in patients with MGUS, with a higher proportion of patients with MGUS (55.3 vs 29.0, p = 0.002) having elevated levels of IgG1 in their serum. Of the 38 patients with MGUS, 21 had an abnormal IgG1 level, 15 had an abnormal IgG2 level, 7 had abnormal IgG3, 5 an abnormal IgG4 level, and 9 patients had more than 1 specific abnormal IgG subclass level.

We compared patients' disease characteristics and manifestations with respect to the presence of elevated levels for each IgG subclass only in the 202 consecutive patients to avoid any bias in the results. Patients with elevated IgG1 subclass levels were more likely to have MGUS (13.1% vs 7.8%, although this did not reach statistical significance; p = 0.23), whereas in the IgG2–IgG4 subtypes the trend suggested lower levels in patients with MGUS. Patients with abnormal IgG1 also had elevated ESR (20.9 vs 11.6, p = 0.003), total protein (73.8 vs 70.7 g/l, p = 0.001), and gammaglobulin levels (11.8 vs 8.7 g/l, p = 0.002), as shown in Table 2. Other disease domains and manifestations including the presence of enthesitis, uveitis, or IBD did not correlate with abnormal levels of IgG1. No difference in treatment was found.

Patients with abnormal IgG2 subclass levels did not exhibit any clinically relevant differences in disease characteristics, manifestations, or treatment compared to patients with a normal IgG2 level. Nor were there differences in the prevalence of a discrete band on serum protein electrophoresis or MGUS.

Patients with abnormal IgG3 levels had a lower active joint count (3.8 vs 6.9, p = 0.02). No difference was noted in any other disease characteristics and manifestations.

No clinical-serological association was found in patients with abnormal IgG4 subclass levels.

DISCUSSION

There have been numerous studies of immunoglobulin isotype levels in psoriasis and PsA, with elevation in IgA being the most consistent finding. However, no correlation was found with disease activity[14]. In this cross-sectional study, we investigated IgG subclass levels, because IgG was shown to be the commonest immunoglobulin isotype in patients with MGUS. We evaluated IgG subclass levels in the sera of 221 patients with PsA, of whom the vast majority were seronegative for RF. Although our analysis revealed that IgG2 was the most common subclass abnormality, the presence of elevation in this subclass was not associated

Table 1. Demographics and disease characteristics of patients based on the presence of MGUS. Data are frequency (%) unless otherwise indicated.

Variable	MGUS Absent, n = 183	MGUS Present, n = 38	p
Age, yrs, mean (SD)	54.7 (12.5)	61.3 (11.5)	0.003
Sex, males	115 (62.8)	24 (63.2)	0.97
Age at diagnosis of psoriasis, yrs, mean (SD)	28.6 (14.5)	28.3 (12.9)	0.88
Age at diagnosis of PsA, yrs, mean (SD)	38.1 (13.8)	38.2 (9.9)	0.75
Disease duration of psoriasis, yrs, mean (SD)	26.0 (14.3)	33.1 (15.8)	0.007
Disease duration of PsA, yrs, mean (SD)	16.6 (12.0)	23.1 (11.7)	0.007
Current PASI score, mean (SD)	2.7 (4.6)	2.0 (2.1)	0.18
Current active joint count, mean (SD)	5.4 (5.9)	11.1 (9.7)	0.04
Current ESR, mean (SD)	12.1 (14.8)	21.5 (26.1)	0.04
Abnormal ESR*	10 (26.3)	22 (12.1)	0.02
Current CRP, mean (SD)	2.8 (1.6)	21.8 (31.1)	0.05
Damaged joint count, mean (SD)	11.2 (11.9)	16.3 (14.8)	0.05
Iritis	7 (3.8)	1 (2.6)	1.0
Iritis ever	23 (12.6)	3 (7.9)	0.58
Iritis active today	1 (0.6)	0 (0)	0.65
IBD	4 (2.2)	1 (2.6)	0.87
IBD ever	4 (2.2)	1 (2.6)	1.0
IBD active today	0 (0)	1 (2.6)	0.17
Enthesitis	18 (9.8)	6 (16.2)	0.25
Enthesitis ever	102 (55.7)	20 (52.6)	0.73
Presence of nail disease	97 (53.0)	20 (52.6)	0.97
Presence of infection within 3 mos of the visit	34 (18.6)	6 (15.8)	0.68
Current NSAID	102 (57.3)	21 (58.3)	0.91
NSAID ever	173 (94.5)	36 (94.7)	1.0
Current DMARD	94 (52.5)	26 (68.4)	0.07
DMARD ever	160 (87.4)	36 (94.7)	0.27
Current biologics	90 (50.6)	15 (42.9)	0.46
Biologics ever	111 (60.7)	21 (55.3)	0.54
IgG1, mean (SD)	649.7 (428.7)	1631.0 (3007.7)	0.06
IgG1 abnormal	53 (29.0)	21 (55.3)	0.002
IgG2, mean (SD)	648.9 (387.9)	572.8 (347.0)	0.26
IgG2 abnormal	93 (50.8)	15 (39.5)	0.20
IgG3, mean (SD)	140.6 (466.6)	146.6 (312.2)	0.94
IgG3 abnormal	41 (22.4)	7 (18.4)	0.59
IgG4, mean (SD)	173.3 (554.0)	81.9 (179.8)	0.07
IgG4 abnormal	40 (21.9)	5 (13.2)	0.23

* Abnormal ESR was defined in males as ≥ 15 mm/h if age < 50 years and ≥ 20 mm/h if age ≥ 50, and in females as ESR ≥ 20 mm/h if age < 50 years and ≥ 30 mm/h if age ≥ 50. MGUS: monoclonal gammopathy of undetermined significance; PsA: psoriatic arthritis; PASI: Psoriasis Area and Severity Index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; IBD: inflammatory bowel disease; NSAID: nonsteroidal antiinflammatory drugs; DMARD: disease-modifying antirheumatic drug.

with any specific disease or serologic differences. We find that interesting, given that IgG2 is produced predominantly in response to capsular antigens, implicating a possible role for bacterial infection with encapsulated organisms in triggering PsA. If such infections play a role, it is likely that they are subclinical because there were no increased reports of preceding infection in this specific subgroup compared to patients with normal levels of IgG2. Notably, this finding is contrary to what was reported in patients with RA who showed predominantly higher levels of IgG1[9], possibly reflecting the different pathogenic mechanisms in these 2 conditions. Cytokines that are involved in the humoral response also play a role in disease pathogenesis. In mouse studies, IFN-γ, a product of Th1 cells, promotes IgG2 switching[15]. Th1 cells have been shown to play a role in PsA pathogenesis and also interact with B lymphocytes, stimulating the production of antibodies for microbial opsonization. Following uptake, various bacteria act on dendritic cells, stimulating production of cytokines including IL-6, IL-1, and IL-23, which are also known to drive the Th17 pathway[16,17,18], thought to be pivotal in the pathogenesis of PsA. Thus, there are potential links that could explain the relationships between PsA, antibody formation, and inflammation.

Of the 4 subclasses, patients with abnormal IgG1 were more likely to have MGUS and abnormal ESR levels. Conversely, patients with MGUS were also most likely to have an abnormal level of IgG1 subclass level, and as expected were older compared to patients without MGUS. In addition, they had not only a longer duration of psoriasis and PsA, but also more active disease and joint damage. As

Table 2. Demographics and disease characteristics of the 202 consecutive patients with normal and abnormal IgG1 levels.

Variable	Mean (SD) or Frequency (%)		p
	Abnormal, n = 61	Normal, n = 141	
Age, yrs	54.2 (13.6)	54.2 (11.1)	0.95
Sex, males	33 (54.1%)	93 (66.0%)	0.11
Age at diagnosis of psoriasis, yrs	30.4 (15.0)	27.6 (13.9)	0.20
Age at diagnosis of PsA, yrs	39.9 (13.2)	37.2 (13.3)	0.19
Disease duration of PsA, yrs	14.3 (10.2)	17.1 (12.4)	0.13
Current PASI score	2.7 (5.0)	3.3 (5.6)	0.55
Current active joint count	5.3 (6.7)	6.4 (6.7)	0.43
Current ESR	20.9 (21.2)	11.6 (14.1)	0.003
Abnormal ESR*	15 (25.0%)	19 (13.5%)	0.046
Damaged joint count	11.9 (13.0)	10.9 (11.5)	0.67
Total protein, g/l	73.8 (6.2)	70.7 (4.3)	0.001
Gammaglobulin, g/l	11.8 (6.7)	8.7 (2.3)	0.002
Presence of ill-defined band in serum protein electrophoresis	5 (9.4%)	10 (7.4%)	0.76
Presence of discrete band in serum protein electrophoresis	9 (17.0%)	13 (9.6%)	0.15
Presence of MGUS	8 (13.1%)	11 (7.8%)	0.23

* Abnormal ESR was defined in males as ≥ 15 mm/h if age < 50 years and ≥ 20 mm/h if age ≥ 50 years, and in females as ESR ≥ 20 mm/h if age < 50 years and ≥ 30 if age ≥ 50 years. MGUS: monoclonal gammopathy of undetermined significance; PsA: psoriatic arthritis; PASI: Psoriasis Area and Severity Index; ESR: erythrocyte sedimentation rate.

mentioned, the finding that immunoglobulin expression is differentially induced by proinflammatory cytokines that mediate the adaptive immune response in PsA supports a role for inflammation in the pathogenesis of MGUS in PsA.

Determination of the IgG subclass concentration in PsA did not seem to add any significant value in identifying specific disease manifestations. However, this study provides insight into the pathological process leading to MGUS in PsA.

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