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Abstract

Background: A small proportion of multiple sclerosis (MS) patients treated with natalizumab develop anti-drug antibodies.

Objective: The objective of this paper is to characterize the anti-natalizumab antibody response and to investigate differences between persistently and transiently antibody-positive patients.

Methods: Screening for anti-natalizumab antibodies was performed using a standardized bridging ELISA. Antibody-positive samples were further analyzed for IgM and IgG1–4 antibodies using ELISA and ImmunoCAP®.

Results: Anti-natalizumab antibodies developed in 57 of 1379 (4.1%) treated patients after a median treatment duration of three months. Of the positive patients, 20 (35%) patients reverted to negative, 19 (33%) patients were confirmed persistently positive and 18 (32%) patients were unconfirmed positive. Significantly higher anti-natalizumab antibody levels were detected in persistently compared to transiently positive patients. A cutoff value predicting persistence of antibodies could be determined with a sensitivity of 0.84 and a specificity of 0.80. IgM and IgG4 antibody levels were significantly higher in persistently compared to transiently positive patients, and IgG1, IgG2 and IgG4 increased significantly over time.

Conclusions: The level of total anti-natalizumab antibodies in a first positive sample can be used to predict patients at risk for persisting antibody positivity. However, neither IgM nor IgG1–4 antibodies could be used to discriminate between transiently and persistently positive patients.

Keywords

Anti-drug antibody, natalizumab, multiple sclerosis

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Introduction

The monoclonal antibody natalizumab (Tysabri®; Biogen Idec/Elan Pharmaceuticals) targets the integrin $\alpha 4 \beta 1$ (very late antigen-4; VLA-4) and is used as a treatment in multiple sclerosis (MS). By binding to VLA-4, natalizumab prevents lymphocytes from crossing the blood-brain barrier and thereby reduces the inflammatory response and the subsequent myelin loss in the brain and spinal cord of MS patients.¹ Treatment with natalizumab efficiently reduces the disease activity and risk of disability progression in patients with relapsing–remitting MS.^{2–7}

Natalizumab seems to be generally well tolerated,^{3,5–7} but a relatively common side effect of protein therapeutics is the development of anti-drug antibodies (ADAs).^{8,9} The humanization of natalizumab, by grafting of the complementarity-determining regions of a murine antibody into a human IgG4 antibody backbone,¹⁰ has probably reduced its immunogenicity compared to other protein therapeutics.¹¹

Even so, antibodies against natalizumab develop in a small proportion of treated patients. In the clinical trials, development of anti-natalizumab antibodies was reported in 9% to 12% of the treated patients.^{6,7} In both trials 6% of the patients became persistently positive for anti-natalizumab antibodies. In recent studies the incidence of anti-natalizumab antibodies has been reported to be 4.5%, with 1.6%

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to 3.5% of the patients becoming persistently antibody positive.^{3,12}

The presence of anti-natalizumab antibodies is related to an increased risk for patients to develop infusion-related adverse events, such as allergic, hypersensitivity and anaphylactic reactions.^{6,7,13,14} Persistently antibody-positive patients also have a reduced clinical effect of natalizumab treatment compared to antibody-negative patients. However, in transiently antibody-positive patients the anti-natalizumab antibodies do not seem to cause a sustained loss of therapeutic efficacy compared with antibody-negative patients.¹³ Discontinuing natalizumab treatment is advised only for patients with two or more consecutive antibody-positive samples taken at least six weeks apart.^{6,13}

The aim of this study was to characterize the anti-natalizumab antibody response in a Swedish cohort of natalizumab-treated patients, and to use the data from the first sample to predict persistency of ADA.

Patients

The study assessed all Swedish natalizumab-treated patients who had been tested at least once before and once after natalizumab treatment start for the presence of anti-natalizumab antibodies between August 2006 and September 2011 ($n_{patients} = 1391$, $n_{samples} = 5425$). Patient information was obtained from the national Swedish MS registry (SMS-registry; <http://www.msreg.net>). Patients were defined as persistently antibody positive if anti-natalizumab antibodies were detected in two or more consecutive samples collected at least six weeks apart. Patients with at least one negative sample following a positive sample were defined as transiently antibody positive, whereas patients without a confirmatory follow-up sample were classified as unconfirmed antibody positive. This study was approved by the Regional Ethical Review Board at Karolinska Institutet.

Information on disease duration, number of relapses and the Expanded Disability Status Scale (EDSS) score before natalizumab initiation was obtained for 888 patients. This group was representative for the whole study group ($n = 1391$) in regard to age at treatment start, gender distribution, treatment duration at first sampling and total antibody response to natalizumab.

Methods

Screening for anti-natalizumab antibodies

All serum samples were screened for the presence of total anti-natalizumab antibodies using a standardized bridging enzyme-linked immunosorbent assay (ELISA) method developed by Biogen Idec (Cambridge, MA, USA); protocol "Assay procedure to determine natalizumab (Tysabri) immunogenicity (CST02-180AP-R.2)" performed as previously described¹³ with minor modifications. Briefly,

control and serum samples diluted 1:10, with or without competing soluble natalizumab, were transferred to 96-well plates coated with 0.25 µg/ml natalizumab. The competing soluble natalizumab (final concentration 100 µg/ml) was added to competition wells in a one-hour pre-incubation step to confirm binding specificity of serum antibodies in the detection wells (i.e. with no competing natalizumab). Captured antibodies against natalizumab were detected with 1 µg/ml biotinylated natalizumab, followed by incubation with streptavidin-horseradish peroxidase. Tetramethylbenzidine (TMB) substrate (Surmodics/BioFX Laboratories, Eden Prairie, MN, USA) was added to develop the color reaction and stopped by addition of 1N H₂SO₄. The optical density (OD) was measured at 450 nm.

Natalizumab and control samples were obtained from Biogen Idec. The controls included high positive quality control 1 (QC1; 3 µg/ml monoclonal antibody 12C4), low positive quality control 2 (QC2; 0.5 µg/ml 12C4) and a negative control (NC) serum.

The results are reported as OD ratios ($OD_{\text{Detection well}} / OD_{\text{Competition well}}$), where the competition well stands for serum pre-incubated with 100 µg/ml natalizumab. The cutoff for positivity was set at an OD ratio of 2.0. OD values of 6.0 were above the detection range, and samples with OD 6.0 in the detection wells were given an OD ratio of 100.

Detection of isotype-specific anti-natalizumab antibodies

Serum samples positive for anti-natalizumab antibodies in the standardized bridging ELISA ($n_{patients} = 69$, $n_{samples} = 80$) were further analyzed for specific isotypes and subclasses of the anti-natalizumab antibodies. The same procedure as described above was used, with the following modifications: IgG1, IgG2 and IgG3 subclasses were detected using biotinylated monoclonal mouse anti-human IgG1 (clone 8c/6-39; 8.4 µg/ml), IgG2 (HP-6014; 0.3 µg/ml) or IgG3 (HP-6050; 2.6 µg/ml) antibodies (all from Sigma-Aldrich, St. Louis, MO, USA). To detect IgM antibodies against natalizumab, plates were coated with 2 µg/ml anti-human IgM antibody (3840-6, Mabtech, Stockholm, Sweden) overnight at 4°C, followed by the standardized protocol described above. Patient sera positive or negative for anti-natalizumab IgM or IgG1–3 antibodies were included as controls on all plates. The coefficient of variation of the inter-assay and intra-assay variability was ≤ 25%. Results are reported as OD ratios as described above.

Since natalizumab is an IgG4 antibody, an isotype-specific assay based on antibody-binding to the whole natalizumab molecule could not be used for detection of IgG4 antibodies. Thus, a modified natalizumab-specific IgG4 assay was developed using the ImmunoCAP® technology

Table 1. Patient characteristics of all patients ($n = 888$) with clinical data available.

	Transiently antibody positive ($n = 13$)	Persistently antibody positive ($n = 12$)	All antibody positive ($n = 37$) ^a	All antibody negative ($n = 851$)	p value ^c
Age at treatment start, years	41 (15–52)	42 (19–59)	38 (15–60)	36 (13–68)	0.40
male/female	2/11	4/8	7/30	260/591	0.15
Disease duration at treatment start, years	4 (0–11)	4 (0–19)	4 (0–19)	4 (0–31)	0.065
Number of relapses before treatment start	3 (0–8)	2 (1–11)	3 (0–11)	3 (0–23)	0.77
EDSS at treatment start	3.5 (0–9)	2.5 (1.5–6.5)	3 (0–9)	3 (0–8.5)	0.56
Treatment duration at first sampling, months	3 (2–6)	3 (2–12)	3 (1–12)	5 (0–31)	0.0047
Total IgG at treatment start, (g/l) ^b	11.1 (8.6–24.0)	11.9 (6.8–14.7)	11.4 (6.8–24.0)	11.3 (4.9–25.4)	0.56

All values are presented as median (min–max) values. EDSS: Expanded Disability Status Scale; ^aincludes transiently, persistently and unconfirmed antibody-positive patients; ^btotal IgG was measured for 32 antibody-positive and 32 antibody-negative patients; ^c p values are reported for the comparison between all negative and all positive patients. No significant differences were observed between transiently and persistently positive patients.

(Phadia, Uppsala, Sweden) based on F(ab')₂ fragments of the antibody. Briefly, natalizumab was cleaved to F(ab')₂ fragments using pepsin degradation and purified using gel filtration. The F(ab')₂ fragments were coupled to experimental ImmunoCAP tests following the standard method of Phadia. Samples diluted 1:100 in ImmunoCAP IgA/IgG Sample Diluent were analyzed for natalizumab-specific IgG4 antibodies on a Phadia® 100 instrument. The assay quantification range was 0.07–30 mg_A/l antigen-specific IgG4 antibody. Based on the mean value + 2 standard deviations of 18 healthy control donors, a cutoff for positivity was set to 0.125 mg_A/l.

Quantification of total IgG

Total IgG concentration was measured in serum samples ($n = 64$) collected before the first infusion of natalizumab. Thirty-two of these patients seroconverted to anti-natalizumab antibody positivity after treatment start, while the other 32 patients remained negative. The two groups were matched for age, disease duration and gender. Total IgG was measured with ELISA using Human IgG ELISA kit (ALP) from Mabtech according to protocol. Briefly, microtiter plates were coated with 2 µg/ml of the monoclonal antibody MT145 overnight. Serum diluted 1:10⁶ and a two-fold dilution IgG standard curve (0.2–50 ng/ml) were added. Bound IgG was detected by the alkaline phosphatase-conjugated monoclonal anti-IgG antibody MT78. Alkaline phosphate yellow (pNPP) from Sigma-Aldrich was used as substrate. The OD was measured at 405 nm.

Detection of rheumatoid factors (RFs)

To investigate the specificity of samples positive in the standardized bridging ELISA before treatment start, RFs (IgM) were analyzed at the Karolinska University Laboratory using a standardized nephelometry method

with a quantification range of 10–600 international units (IU)/ml, with a cutoff for positivity of 20 IU/ml.

Statistics

All statistical analyses were done using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). The Kruskal-Wallis test with Dunn's multiple comparison test was used for comparison between three groups. The Mann-Whitney test and Fisher's exact test were used for comparisons between two groups. Wilcoxon matched-pairs signed rank test was used when comparing antibody change over time. A p value below 0.05 was considered significant.

Results

Frequency of ADAs during natalizumab treatment

Of all natalizumab-treated patients with a negative serum sample before treatment start, anti-natalizumab antibodies were detected in 4.1% (57/1379) of the patients during treatment. Of the positive patients, 96% (55/57) were positive already in their first collected sample after treatment start with a median time of three months to seroconversion (Table 1). During further investigation of the 57 antibody-positive patients, 20 patients reverted to negativity in a later sample (transiently positive), 19 patients were positive in two consecutive samples (persistently positive) and 18 patients were unconfirmed positive.

Neither demographic, clinical features nor the amount of total IgG were observed to differ significantly between antibody-positive and antibody-negative patients, except for antibody-negative patients being treated with natalizumab for a longer time than antibody-positive patients when the first sample for antibody analysis was collected (Table 1).

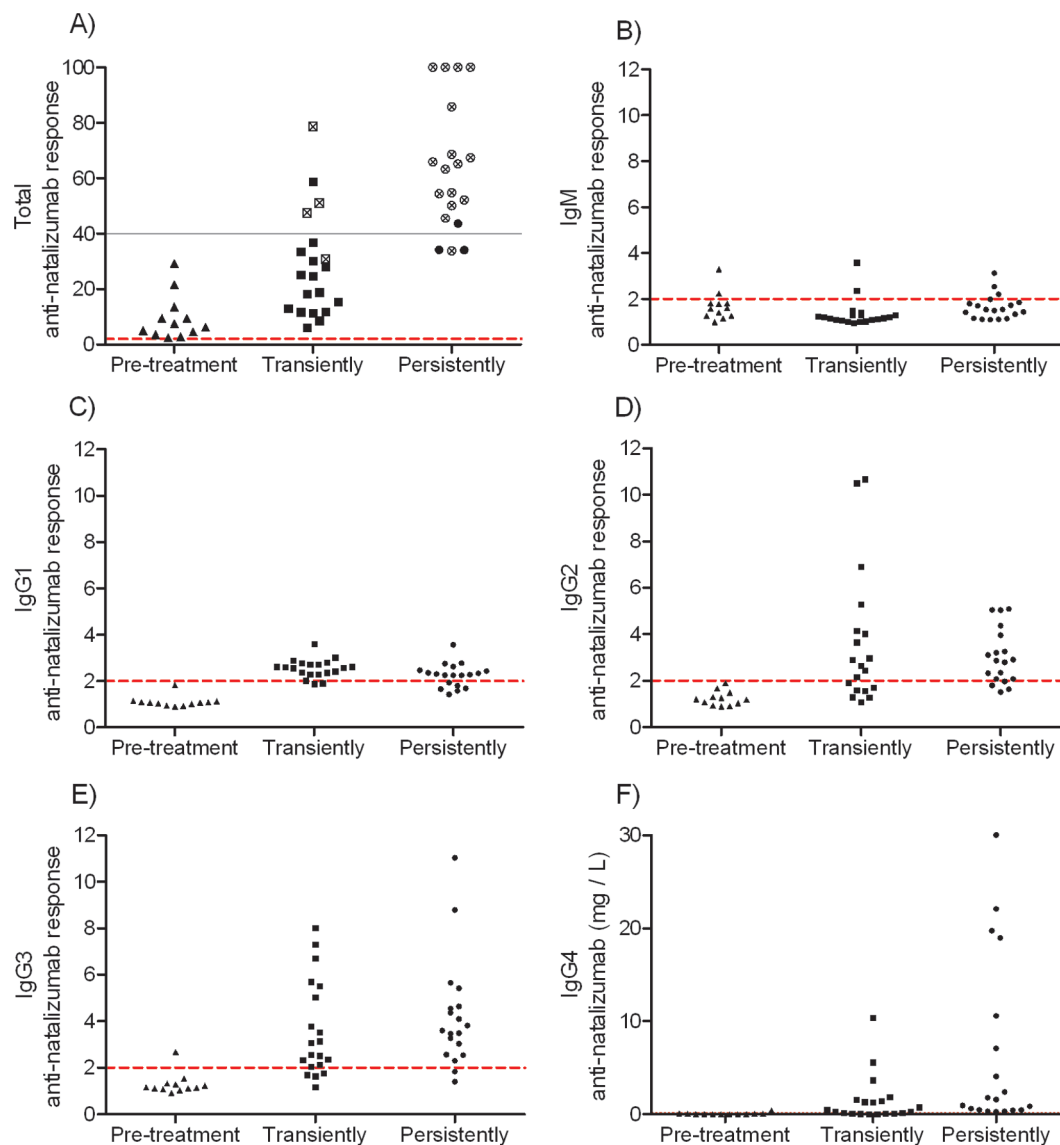


Figure 1. Total antibody response and isotype characterization of the anti-natalizumab antibodies. “Pre-treatment”: positive samples collected before treatment start ($n = 12$); “Transiently” and “Persistently”: The first positive samples after treatment start from transiently ($n = 20$) and persistently ($n = 19$) antibody-positive patients. a) Total anti-natalizumab response measured by a standardized bridging enzyme-linked immunosorbent assay (ELISA). Samples with an antibody concentration $\geq 3 \mu\text{g/ml}$ are shown as crossed squares or circles. Samples with an antibody concentration between 0.5 and $3 \mu\text{g/ml}$ are shown as filled shapes. The grey line indicates the predictive value for antibody persistence. Isotype-specific ELISAs were used to detect anti-natalizumab b) IgM, c) IgG1, d) IgG2 and e) IgG3 antibodies. In Figure 1a–e, results are presented as optical density (OD) ratios ($\text{OD}_{\text{Detection well}}/\text{OD}_{\text{Competition well}}$). The dotted lines illustrate the cutoff for positivity. Samples with an $\text{OD}_{\text{Detection well}}$ value of 6.0 were given the OD ratio 100. In Figure 1f, IgG4 antibodies were measured using ImmunoCAP®; results are presented as mg_A/l of antigen-specific IgG4 antibody. One sample was above the quantification range of the assay and was given the value $30 \text{ mg}_A/\text{l}$.

A few patients have an immune response against natalizumab before treatment start

Unexpectedly, 0.9% (12/1391) of all patients were defined as positive in their sample collected before the first infusion of natalizumab. Of these 12 patients, only three remained positive after treatment start. All positive pre-treatment samples had a very low reactivity against natalizumab (Figure 1A), which was significantly lower compared to

samples from persistently and transiently positive patients ($p < 0.001$ and $p < 0.05$, respectively). All pre-treatment samples were negative for IgM RF.

Differences in antibody response between transiently and persistently antibody-positive patients

To address the question of whether the transiently and persistently positive groups were different in their antibody

Table 2. Differences in antibody response between transiently and persistently antibody-positive patients.

Anti-natalizumab response ^a	Transiently antibody-positive patients, <i>n</i> = 20		Persistently antibody-positive patients, <i>n</i> = 19		<i>p</i> value
	% positive	Antibody response	% positive	Antibody response	
Total	100	25 (6–79)	100	63 (34–100)	< 0.0001
IgM	10	1.2 (1.0–3.6)	16	1.5 (1.1–3.1)	0.0073
IgG1	90	2.6 (1.9–3.6)	68	2.3 (1.4–3.6)	0.021
IgG2	65	2.8 (1.1–10.7)	79	2.9 (1.5–5.1)	0.92
IgG3	80	2.8 (1.2–8.0)	89	3.6 (1.4–11.0)	0.28
IgG4 (mg _A /l)	70	0.4 (< 0.07–10.4)	100	1.6 (0.3–30)	0.011

All values for antibody response are presented as median (min–max) values; ^atotal, IgM and IgG1–3 antibody responses are presented as optical density (OD) ratios (OD_{Detection well}/OD_{Competition well}); samples with OD_{Detection well} values of 6.0 were given an OD ratio of 100; IgG4 antibody response analyzed using ImmunoCAP® is presented as mg_A/l; samples above the quantification range of the assay were given the value 30 mg_A/l.

response against natalizumab, we first analyzed the total response to natalizumab measured by a standardized bridging ELISA. Persistently positive patients had a significantly stronger immune response than transiently positive patients ($p < 0.0001$) (Table 2). As shown in Figure 1a, 80% (16/20) of the transiently positive patients had an antibody response between the concentration of QC2 and QC1 (0.5–3 µg/ml), while 84% (16/19) of the persistently positive patients had a value equal or above QC1 (3 µg/ml). Using an OD ratio of 40 as cutoff value (Figure 1a), the standardized bridging ELISA had a positive predictive value of 0.80 for predicting persisting positivity with a sensitivity of 0.84 and a specificity of 0.80.

To further investigate the difference between the antibody-positive groups, anti-natalizumab IgM and IgG1–4 antibodies were measured in all positive patients' first positive sample (Figure 1b–f). Persistently positive patients had significantly higher levels of anti-natalizumab IgM ($p = 0.0073$) and IgG4 ($p = 0.011$) but lower levels of IgG1 antibodies ($p = 0.021$) compared to transiently positive patients (Table 2). The levels of all four subclasses of anti-natalizumab IgG antibodies were significantly lower in the positive serum samples collected before treatment start compared to the first positive sample of the transiently and persistently positive patients ($p < 0.01$ – 0.001).

The anti-natalizumab antibody pattern change over time

The change in antibody repertoire over time was investigated by comparing samples from persistently positive patients collected at different time points. The first sample was collected after 2.0–3.8 months of treatment and the second after 4.8–6.6 months of treatment ($n_{patients} = 11$). Between these time points, the total anti-natalizumab response increased in all patients except in the two with the lowest response in their first sample (Figure 2a). The anti-natalizumab IgG1, IgG2 and IgG4 responses increased significantly ($p = 0.0010$, $p = 0.0049$ and $p = 0.0020$, respectively) over time (Figure 2b–f).

Negative samples collected five to 11 months after a positive sample ($n = 18$) were analyzed for the presence of anti-natalizumab-specific IgG4 antibodies. These negative samples contained < 0.07–0.51 mg_A/l anti-natalizumab-specific IgG4 antibodies, where only four of the 18 samples had IgG4 antibodies above the positive cutoff point of 0.125 mg_A/l.

Levels of IgG subclasses decrease after treatment stop

The evolution of anti-natalizumab antibodies after treatment discontinuation was investigated in one persistently positive patient with two additional positive serum samples collected five and 17 months after the last natalizumab infusion. Five months after the last natalizumab infusion, IgG1–3 antibodies were approximately at the same level as during treatment while IgG4 concentration had decreased from 23 mg_A/l to 2.5 mg_A/l. At 17 months post-treatment, only IgG1 levels were unchanged, with an approximate OD ratio of 3. However, IgG2 levels had decreased from OD ratio 5.2 to 2.8, IgG3 levels from 2.4 to negative, and IgG4 concentration had dropped to 0.17 mg_A/ml. The patient was negative for anti-natalizumab IgM antibodies in all samples.

Discussion

Like other protein-based therapies, treatment with the monoclonal antibody natalizumab can induce immunogenicity in treated patients, resulting in the formation of ADAs and loss of clinical effect of the drug.¹³ In this study the results from all patients tested for anti-natalizumab antibodies in Sweden over a period of five years were evaluated to characterize the anti-natalizumab antibody response. Antibodies against natalizumab were detected in only 4.1% of the patients during treatment, with a median treatment time of three months to the first positive sample, thus confirming the early development of antibodies against natalizumab previously shown by others.^{14,15} The somewhat lower proportion of positive patients than reported in the first clinical trials is more in line with results from recent studies with

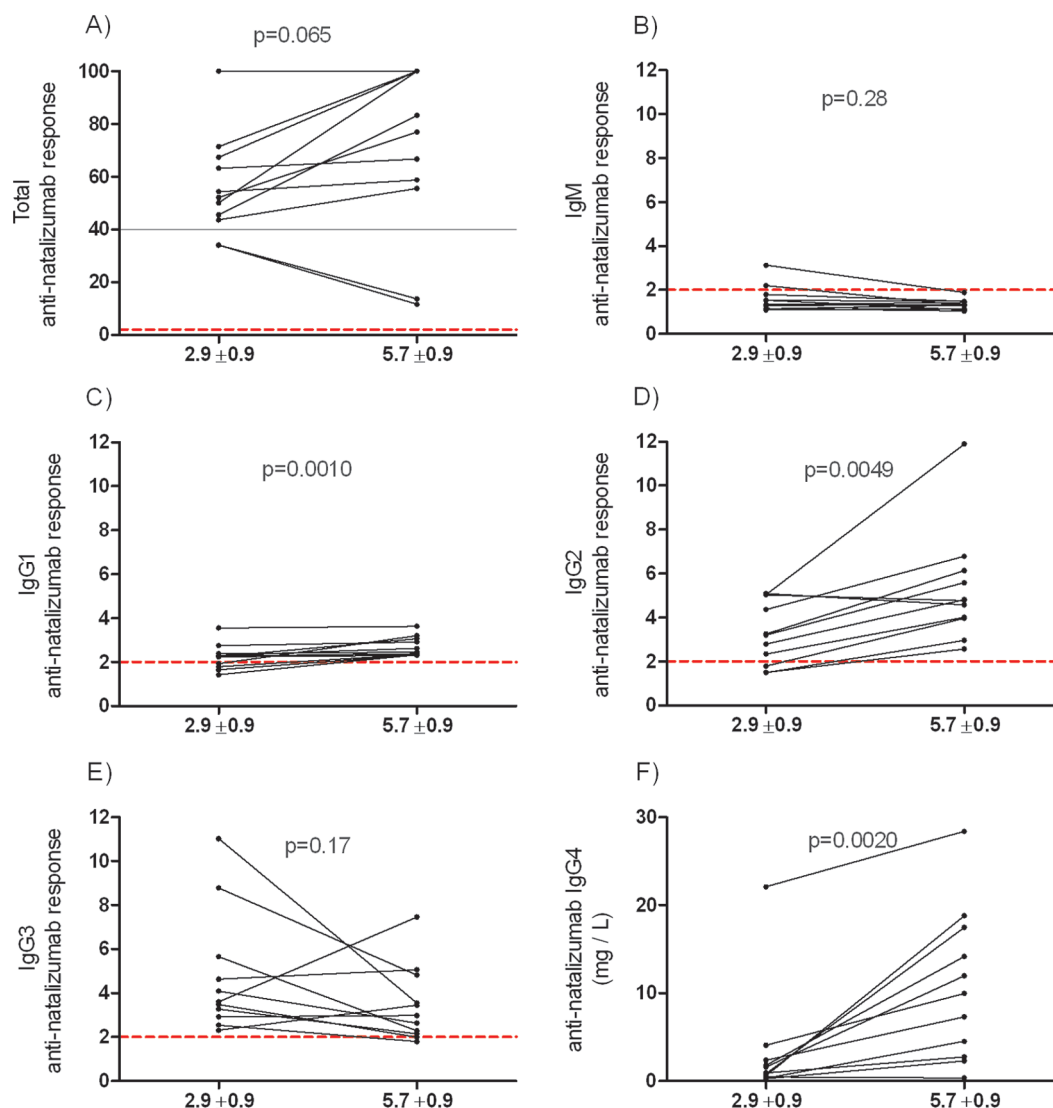


Figure 2. Change in antibody repertoire in persistently positive patients ($n = 11$) over time. The first samples were collected after 2.0–3.8 months of treatment and the second samples after 4.8–6.6 months of treatment, with at least six weeks between one patient's samples. a) Total anti-natalizumab response measured by a standardized bridging enzyme-linked immunosorbent assay (ELISA). The grey line indicates the predictive value for antibody persistence. ELISAs were used to measure anti-natalizumab b) IgM, c) IgG1, d) IgG2 and e) IgG3 antibodies. a–e) The results are presented as optical density (OD) ratios ($OD_{\text{Detection well}}/OD_{\text{Competition well}}$). Samples with $OD_{\text{Detection well}}$ values of 6.0 were given the OD ratio 100. The dotted lines illustrate the cutoff for positivity. f) IgG4 antibodies were measured using ImmunoCAP®, results are presented as mg_A/l of antigen-specific IgG4 antibody.

larger groups of patients reporting a frequency of antibody development in less than 5% of the patients.^{3,12} One explanation for the low proportion of antibody-positive patients in this study might be due to the observed difference in time to first sampling between antibody-positive and antibody-negative patients. If the first sample is collected later than six months after treatment start, patients classified as antibody negative might have been positive earlier but converted to negativity, thereby underestimating the total antibody positivity. Another explanation could be that patients who develop anti-natalizumab antibodies discontinue treatment before first sampling because of hypersensitivity or other infusion-related adverse events.

Positivity for anti-natalizumab antibodies was unexpectedly found in almost 1% of all pre-treatment samples. However, for most of these patients the reactivity against natalizumab disappeared when treatment was initiated. This reactivity could not be explained by non-specific interference of IgM RFs,¹⁶ i.e. low-affinity antibodies binding to the Fc part of IgG, since all samples were negative for RFs. However, we cannot exclude the possible presence of other heterophilic antibodies,¹⁷ non-specific anti-hinge antibodies¹⁸ or antibodies against carbohydrates¹⁹ leading to false-positive results in the bridging ELISA.

To investigate whether the antibody response against natalizumab differs between persistently and transiently

positive patients, the positive samples were further characterized. Persistently positive patients were found to have considerably higher total antibody levels against natalizumab already in their first positive sample compared to transiently positive patients. Our observation of a higher antibody production against natalizumab in persistently positive patients is in accordance with recently reported results showing that persistently positive patients have higher levels of anti-natalizumab antibodies than transiently positive patients already at three months after treatment initiation, and that the titer level at month three can be predictive of which patients are likely to develop persistent antibodies and therefore need to discontinue therapy.²⁰ Compared to performing a titration of each sample, as described by Jensen et al.,²⁰ we used the results from the screening ELISA assay developed by Biogen Idec to assess the antibody response and to distinguish transiently and persistently positive patients from each other. Using an OD ratio ($OD_{\text{Detection well}}/OD_{\text{Competition well}}$) of 40 as a threshold, this method classified 84% of the persistently positive and 80% of the transiently positive patients correctly. Although the screening method used in this study is somewhat less specific than the titration method described by Jensen et al., screening of the samples is less time consuming and expensive compared to titration of the samples. An additional advantage is that the result from an already established screening assay used in the routine laboratory can be used to predict antibody persistence.

Significant differences in the levels of natalizumab-specific antibodies of different isotypes were observed between persistently and transiently positive patients. Although the levels of anti-natalizumab IgM and IgG4 antibodies were higher in persistently positive patients and transiently positive patients had higher IgG1 antibody levels, none of these antibody isotypes could be used to predict persisting antibody positivity.

Class switching to IgG4 is generally considered to occur after long-term exposure to antigens. In this study, IgG4 antibodies against natalizumab were detected in samples taken already between three and six months after treatment start. This rather unexpected early development of an IgG4 response against a protein therapeutic has been noted before in a longitudinal study on ADA against the monoclonal antibody adalimumab in rheumatoid arthritis patients, where IgG4 responses to adalimumab were observed already after 16 weeks in three out of nine patients.²¹

The higher IgG4 levels and the lower IgG1 levels observed in persistently antibody-positive patients compared to transiently positive patients is in line with results from factor VIII (FVIII)-treated hemophilia A patients. IgG4 antibodies were more frequent in patients with high titers of anti-FVIII antibodies whereas IgG1 antibodies were more prominent in low titer-positive patients.²² We could observe a significant increase in IgG1, IgG2 and IgG4 over time. A switch to IgG4 and a progressively increasing

titer of ADAs of the IgG4 subclass has been reported during long-term treatment with other protein-based therapeutics. Interferon beta (IFN β)-treated MS patients positive for neutralizing antibodies (NAbs) against IFN β have higher levels of IgG4 antibodies compared to patients with non-neutralizing antibodies to IFN β .^{23,24} When investigating the evolution of anti-natalizumab antibodies following treatment discontinuation in one persistently positive patient, low levels of IgG1, IgG2 and IgG4 antibodies were still detectable after 17 months following treatment discontinuation. This can be compared with patients who develop NAbs against recombinant IFN β , in which antibody titers can persist for several years after discontinued IFN β therapy.²⁵

IgG4 may undergo Fab arm exchange *in vivo*, resulting in monovalent antibodies.²⁶ If this occurs, the bridging ELISA used in this study would be unable to detect these monovalent antibodies, thus resulting in false negativity. To ensure that the negative follow-up samples from transiently positive patients did not contain anti-natalizumab IgG4 antibodies, these samples were analyzed using an experimental natalizumab F(ab')₂ fragment ImmunoCAP test in the ImmunoCAP Specific IgG4 assay. Since all of these samples were negative or very low positive for IgG4 antibodies, false negativity due to inability of the bridging ELISA to detect anti-natalizumab IgG4 antibodies does not seem to explain the observed loss of antibody reactivity of the transiently positive patients.

Another potential problem with the assay used in this study could be the inability to detect antibodies that form immune complexes with the drug.²⁷ The reversion of transiently antibody-positive patients to antibody negativity, which is thought to be a result of tolerance to natalizumab, could potentially be a result of the increasing natalizumab concentration in serum leading to formation of undetectable ADA-drug complexes. However, the increasing concentration of natalizumab and the restored treatment efficacy over time in transiently positive patients¹³ indicates that patients do revert to antibody negativity, or at least to lower antibody levels that do not have a significant clinical effect.

With the results from the standardized bridging ELISA described by Biogen Idec used in the present study, it is possible to identify patients with high risk for persisting antibodies already after three months of treatment. Thus, repeated analysis of antibodies would be required only for those with a high antibody response and/or under special circumstances, e.g. when hypersensitivity, other infusion-related adverse events or treatment failure is suspected.

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Conflict of interest

M.L. has received unrestricted research grants from Biogen Idec and Sanofi-Aventis as well as payment for lectures from Biogen Idec. E.E. has received unrestricted research grants from Merck Serono. C.H. and R.M. report no conflict of interest. T.O. has received unrestricted research grants, advisory board and lecture honoraria from the following companies: Biogen Idec, Novartis, Merck and Genzyme. Academic grants have been received from the Swedish research council, the Swedish Brain Foundation, the Söderberg Foundation and Knuta and Alice Wallenbers Foundation. J.H. received honoraria for serving on advisory boards for Biogen Idec and speaker's fees from Biogen Idec, Merck-Serono, Bayer-Schering, Teva and Sanofi-Aventis. He has served as principal investigator (PI) for projects sponsored by, or received unrestricted research support from, Biogen Idec, Merck-Serono, TEVA, Novartis and Bayer-Schering. A.F.-H. has received consultancy fees from Janssen Pharmaceutica N.V., payment for lectures from Biogen Idec, and research grants from Biogen Idec and Sanofi Aventis.

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