Online Supplemental Material for

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A. Biacore analytical techniques. (For space considerations, the detailed description of the Biacore analysis of the GPI-antibody interactions was not included in the article.) Surface plasmon resonance (SPR) measurements were performed with a BIAcore 2000 instrument (Biacore AB) at 25°C in 10 mM Hepes, 150 mM NaCl, 3.4 mM EDTA, 0.005% surfactant P20, pH 7.4 (HBS) with research grade CM5 sensor chips. Rabbit anti–mouse Fc (RAM) or anti-GPI mAbs were immobilized on the sensor chip via amino groups using standard procedures (Lofas, S., and B. Johnsson. 1990. *J. Chem. Soc. Chem. Commun.* 21:1526–1528). The Abs (100 μg/ml, 10 mM acetate, pH 5) were immobilized at different densities depending on the assay performed (see below). After each binding experiment, the Ab-biosensor surface was regenerated with a pulse (1 min) of acid solution (25 mM HCl for the RAM surface, or 10 mM glycine, pH 2.2, for anti-GPI mAb surfaces).

To generate kinetic binding data, we captured anti-GPI mAbs (100 RU) on the RAM surface to obtain homogeneously immobilized mAb surfaces. These mAb surfaces were washed for 10 min with HBS prior to the injection of GPI until the baseline signal was stable. The flow rate was increased to 30 μl/min, and the GPI was injected for 2 min. The GPI samples (five different concentrations ranging from 9 to 150 nM) were injected in parallel over three mAb surfaces and on an mAb-free surface to control for refractive index change and nonspecific binding. After binding, the sample was replaced by HBS, and GPI dissociation was monitored for 30 min. Replicates and randomized GPI samples and buffer blank injections were performed. Data from the reference surface was subtracted from data from the reaction surface, and the response of the blank injection was subtracted from the entire data set ("double referencing "; Myszka, D.G. 1999. *J. Mol. Recognit.* 12:279–284). The association and dissociation rate constants were analyzed with the BIAevaluation 3.0 Software (Biacore AB). The sensorgrams obtained at different concentrations were analyzed simultaneously using global fitting in combination with numerical integration calculations (Karlsson, R., and A. Falt. 1997. *J. Immunol .Methods.* 200:121–133). Data were fitted to 1:1 Langmuir binding or to two-state conformational change models.

For the two Ab assays, the capturing anti-GPI mAb1 was directly immobilized on the sensor chip at high density (3 to 10 kRU). GPI (2 or 20 nM) was injected for 10 min. The mAb-GPI complexes were washed for 5 min, leaving approximately 30 to 90 RU of GPI on the chip as stable mAb1-GPI complexes. As the detected response is proportional to the protein mass, each mAb–antigen binding event can be quantitated and the stoichiometry of the complex can be calculated as the molar ratio (MR) of the reactant by the following relationship : (MR = [RUmAb/ RUAg] 3 [MWAg/MWmAb]) (Scalice, E.R., D.J. Sharkey, and J.L. Daiss. 1994. *J. Immunol. Methods.* 172:147–163). To measure dissociation kinetics from trimolecular complexes, small quantities of GPI (0.4–0.5 fmol; 30 to 45 RU) were captured on anti-GPI mAb1 surfaces to avoid mass transport limitations and to limit as much as possible avidity effects due to the bivalency of mAb2. Three different concentrations of anti GPI mAb2 (ranging from 2 to 40 nM) were then injected for two minutes over the GPI-mAb1 complexes. Control injection of mAb2 in the absence of captured GPI and buffer blank injection were subtracted. Dissociation of mAb2–GPI-mAb1 complex was monitored during the first 600 s of the post injection phase. The dissociation phases of sensorgrams obtained at different concentrations were fitted to a 1:1 Langmuir dissociation model using the BIAevaluation 3.0 software.

Table S1. V_H and V_L gene usage by anti-GPI mAbs

mAb[§]	Isotype	V_H gene* family usage	V_H germline gene accession number	Mutations[+]		V_k gene* family usage	V_k germline gene accession number	Mutations[+]	
				Repl.	Silent			Repl.	Silent
1.8	IgG1	V1(S20)	X00160	13	5	Vk1(S1)	D00080	1	2
1.24	IgG1	V1(S57)	D13201	21	10	Vk4(S2)	K00884	11	6
2.99	IgG1	V1(S53)	X03571	6	2	Vk2(S2)	K02160	4	-
2.67	IgG2b	V1(S20)	X00160	4	5	Vk10(S3)	M54904	2	-
2.56	IgG2b	V1(S20)	X00160	2	1	Vk2(S7)	M80410	-	1
6.121	IgG1	V1(S20)	X00160	12	6	Vk1(S4)	M28132	3	1
6.149	IgG1	V14(S1)	X03571	10	8	Vk16(S1)	M13833	3	1
6.96	IgG1	V14(S1)	X03571	9	6	Vk10(S2)	M54903	2	3
6.65	IgG1	V1(S12)	J00507	32	4	lambda			

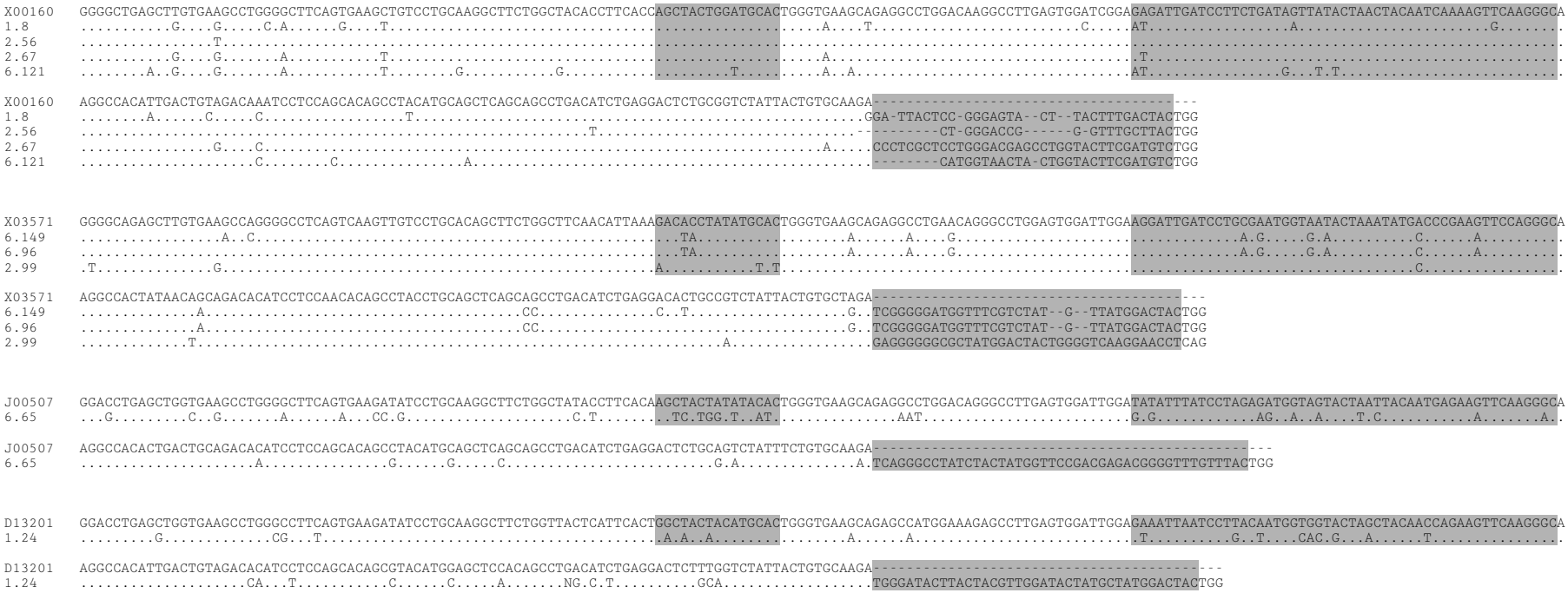
[§] The mAbs have been grouped according to their mouse of origin, indicated by the first number (fusions 1, 2 or 6 - see Table I).

⋆Assignment of the V_H and V_k gene family usage was performed by the IMGT/DNAplot program (IMGT, http://imgt.cines.fr:8104). The IGHV subgroup designations are according to Honjo et al. (1995) and the nomenclature of the IGHV and IGKV is that adopted by the IMGT.

[+]Differences from the germline sequence taken as a reference. As the mAbs originate from a (B6 x NOD)F1 hybrid background, it is not possible to formally assign the differences to interstrain allotypic variation or to somatic mutation.

⋆⋆The V_H and V_L sequences were compared with the germline genes and the percentages of identity were calculated using the GAP program (GCG version 9.1, Genetics Computer Group, Madison,WI)

B. IgG heavy chain sequences for the different hybridomas – Nucleotide sequences



The sequences were obtained by RT-PCR amplification from hybridoma RNA, using degenerate amplification primers. They are aligned in comparison to the closest germline database sequence (see Table S1). The CDR regions are shaded.

IgG heavy chain sequences for the different hybridomas – Protein sequences

Each IgH sequence is compared to the closest database homolog (as of 12/2001; see Table S1). The CDR regions are shaded.

IgG Light chain sequences for the different hybridomas – Protein sequences

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D00080    PLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVP--
1.8       .............M..................S.........................................................FT

M80410    PVTLGTSASISCRSSKSLLHSDGITYLYWYLQRPGQSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLRISRVEAEDVGVYYCAQMLEFP--
2.56      ............................................................................................YT

M54904    LSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKLP--
2.67      ..V..........F.....................................................................YTF

K02160    LAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYLASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNNEDP--
2.99      .....R.....................DT.....................R.....................................WT

M28132    SLPVSLGDQASISCRSSQSLVHSNGNTYLYWYLQKPGQSPKLLIYRVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCFQGTHVP--
6.121     ..............K.....................................IF...................HT

K00884    MAASLGQKVTMTCSASSSVSSSYLHWYQQKSGASPKPLIHRTSNLASGVPARFSGSGSGTSYSLTISSVEAEDDATYYCQQWSGYP--
1.24      .S..P.E......R.............P.S...LW.FS.....................................A.....CDSS.YT

M13833    SPSYLPAAPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGNGSGTDFTLTISSLEPEDFAMYYCQQHNEYPWT
6.149     .....A.S.........R.....................................S.....................

M54903    TTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQDSKHP--
6.96      S.....................................................T...A..................H.VITL.WT
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Each kappa chain sequence is compared to the closest database homolog (as of 12/2001). The CDR regions are shaded.