

Supporting Online Material Manuscript 1111781

Supporting Materials and Methods

Monoclonal Antibodies. MAbs 2F5, 2G12, and 4E10 were produced as described (1-3). IgG1b12 (4) was the generous gift of Dennis Burton, Scripps Institute, La Jolla, CA. MAb 447-52D (5) was obtained from the AIDS Reagent Repository, NIAID, NIH. The remainder of the human MAbs in Table 1 were produced from HIV-1 infected subjects and used as described (6,7). Anti-2F5 idiotype MAb 3H6 was made and used as described (8).

Autoantibody Assays. For all assays a wide dose range of human MAbs were tested. Since clinically significant autoantibody concentrations range from ~100 ug/ml to ~50 mg/ml (9,10), we used autoantibody levels of 150 ug/ml for most assays and as well performed a wide range of titers. An anti-cardiolipin ELISA was used as described (11,12). A similar ELISA was adapted for assay of MAb reactivity to phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin (all purchased from Sigma, St. Louis, MO.). The luminex AtheNA Multi-Lyte ANA Test (Wampole Laboratories, Princeton, NJ) was used to test for MAb reactivity to SS-A/Ro, SS-B/La, Sm, ribonucleoprotein (RNP), Scl-70, Jo-1, double stranded (ds) DNA, centromere B, and histone. MAb concentrations assayed were 150 ug, 50 ug, 15 ug, and 5 ug/ml. Ten ul of each concentration (1.5 ug, 0.5 ug, 0.15 ug, and 0.05 ug, respectively per assay) were incubated with the luminex fluorescent beads and the test performed per manufacturer's specifications. Values in Table 1 are results of assays with 0.5 ug added

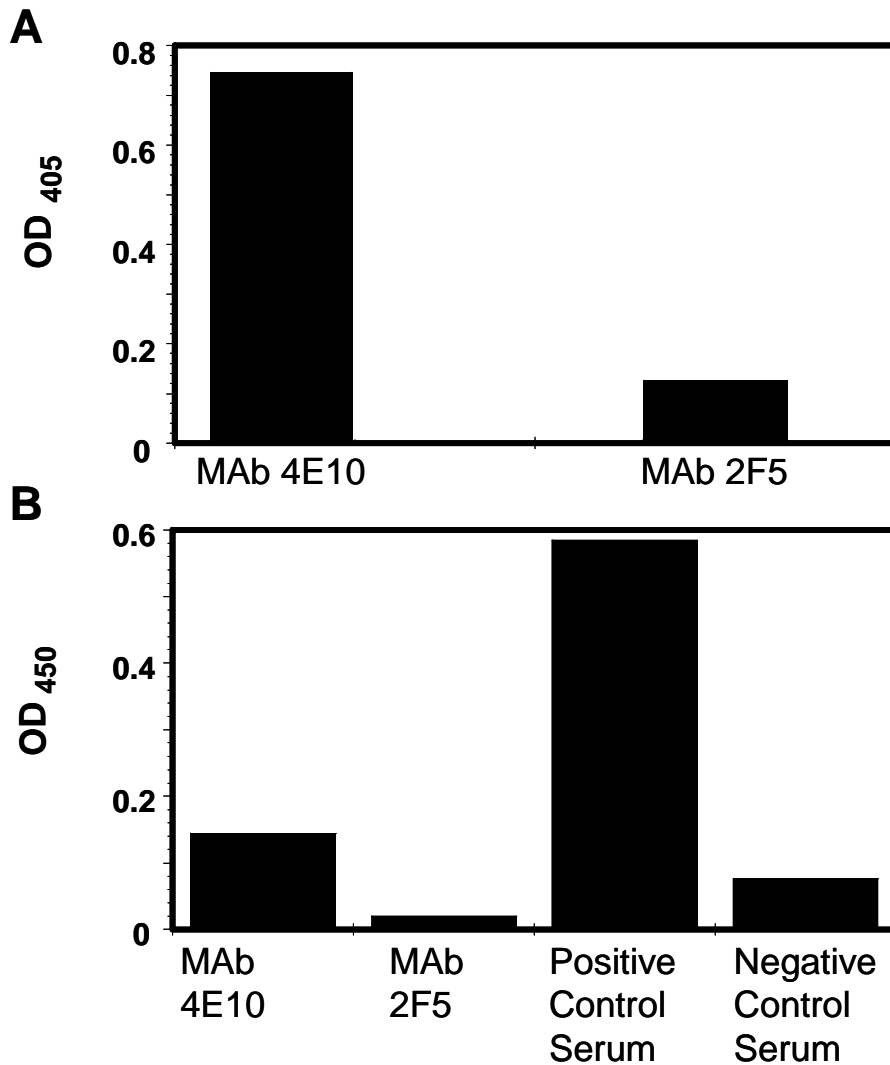
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per test. In addition, an ELISA for SS-A/Ro (ImmunoVision, Springdale, AR) and dsDNA (Inova Diagnostics, San Diego, CA) was also used to confirm these autoantigen specificities. Reactivity to HIV-1 negative human epithelial HEp-2 cells was determined by indirect immunofluorescence on HEp-2 slides using Evans Blue as a counterstain and FITC-conjugated goat anti-human IgG (Zeus Scientific, Raritan N.J.). Slides were photographed on a Nikon Optiphot fluorescence microscope. Regarding Figure 1, kodachrome slides were taken of each MAb binding to HEp-2 cells at a 32 second exposure, and the slides scanned into digital format. Panels A-D were then uniformly adjusted with Photoshop 7.01 to most closely represent the original kodachrome slides with identical adjustment to Panels A-D. Rheumatoid factor was performed by nephelometry (Dade Behring, Inc (Newark, DE). Lupus anticoagulant assay was performed by activated partial thromboplastin (aPTT) and dilute Russell viper venom testing, as described (13). Forty ul of 1 mg/ml of 2F5, 4E10 and control MAbs were added to pooled normal plasma (final MAb concentration, 200 ug/ml) for lupus anticoagulant assay. Anti- β 2 glycoprotein-1 assay was an ELISA (Inova Diagnostics, Inc.) as was anti-prothrombin assay (Prothrombin from Sigma). Serum antibodies to dsDNA, SS-A/Ro, SS-B/La, Sm, RNP, and histone occur in patients with SLE; serum antibodies to centromere B and scl-70 (topoisomerase I) are found in patients with systemic sclerosis; and antibodies to Jo-1 are found in association with polymyositis (14).

Legend to Supporting Online Figure S1

Assay of MAbs 2F5 and 4E10 with Prothrombin and β 2 glycoprotein-1 in ELISA. Panel A shows that MAb 4E10 but not 2F5 reacted with prothrombin in ELISA, while Panel B shows that neither MAb 4E10 nor 2F5 reacted well with β 2 glycoprotein-1 in ELISA. In the prothrombin assay, data reported are at 37.5 ug/ml of 2F5 and 4E10 MAbs; in the β 2 glycoprotein-1 ELISA, data reported are at 150 ug/ml of both MAbs.

Supporting Online Figure 1



Supporting Online Table S1

Table S1. Lupus anticoagulant activity assay of 2F5 and 4E10 MAbs.

Sample	aPTT <i>sec</i>	aPTT mix* <i>sec</i>	DRVVT ratio	Confirmatory ratio
Normal plasma	25.7	25.4	1.000	1.051
Normal plasma + PBS	29.4	-	1.000	1.018
Normal plasma + 17b	29.0	30.3	1.011	1.000
Normal plasma + 7B2	29.0	29.7	0.987	0.989
Normal plasma + 2F5	28.9	29.6	1.093	1.031
Normal plasma + 4E10	39.0	39.5	1.391	1.341
Lupus anticoagulant control	93.2	68.5	2.432	2.303
<i>Normal values</i>	21.1-32.1	21.1-32.1	<1.2	<1.2

* 50:50 mix of each plasma sample with an equal volume of normal plasma with repeat aPTT testing performed. MAbs 17b and 7B2 are negative control human anti-gp120 and gp41 HIV-1 Env MAbs that are not cardiolipin MAbs. MAbs 4E10 and 2F5 as well as control human MAbs 7B2 and 17b were tested at 200 ug/ml for lupus anticoagulant activity by activated partial thromboplastin (aPTT) and dilute Russell viper venom testing (DRVVT), as described (13). In two separate assays, MAbs 2F5, 17b and 7B2 had no lupus anticoagulant activity, while MAb 4E10 had significant lupus anticoagulant activity. Specifically, addition of MAb 4E10 to normal plasma prolonged

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the aPTT which did not correct when mixed with normal plasma, and also prolonged the DRVVT and confirmatory ratio consistent with a lupus anticoagulant. Data shown are representative of two experiments performed.

Supporting References

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