Rapid Monoclonal Antibody Generation Via Dendritic Cell Targeting *In Vivo*

JODY D. BERRY,*1 ALEXEI LICEA,*1,2 MIKHAIL POPKOV,1 XOCHITL CORTEZ,2 ROBERTA FULLER,1 MARIKKA ELIA,1 LISA KERWIN,1 DIANE KUBITZ,1 and CARLOS F. BARBAS, III1

**ABSTRACT**

Dendritic cells (DC) are the professional antigen-presenting cells of the immune system. Previous studies have demonstrated that targeting foreign antigens to DC leads to enhanced antigen (Ag)-specific responses *in vivo*. However, the utility of this strategy for the generation of MAbs has not been investigated. To address this question we immunized mice with IgG-peptide conjugates prepared with the hamster anti-murine CD11c MAb N418. Synthetic peptides corresponding to two different exposed regions of DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN), a human C-type lectin, were conjugated to N418 using thiol-based chemistry. The N418 MAb served as the targeting molecule and synthetic peptides as the Ag (MAb-Ag). A rapid and peptide specific serum IgG response was produced by Day 7 when the synthetic peptides were linked to the N418 MAb, compared to peptide co-delivered with the N418 without linkage. Spleen cells from N418-peptide immunized mice were fused on Day 10, and three IgG1/k monoclonal antibodies (MAbs) were selected to one of the peptide epitopes (MID-peptide). One of the MAbs, Novik 2, bound to two forms of recombinant DC-SIGN protein in enzyme-linked immunosorbent assay (ELISA), and was specifically inhibited by the MID-peptide in solution. Two of these MAbs show specific binding to DC-SIGN expressed by cultured human primary DC. We conclude that *in vivo* DC targeting enhances the immunogenicity of synthetic peptides and is an effective method for the rapid generation of MAbs to predetermined epitopes.

**INTRODUCTION**

Antibodies are one of the main effector arms of the specific immune system, and are the only one capable of completely preventing an infection.1 The exquisite specificity of antibodies to individual epitopes makes them desirable as therapeutics and as targeting domains to host proteins. Many methods for improving the immunogenicity of synthetic and recombinant vaccine candidates have been evaluated. Some of these methods include: Tandem co-delivery of T-cell epitopes2; changing epitope multiplicity, orientation, or polarity3−5; conformationally optimized peptides6; route of immunization7; and MAP-peptide multimer technique.8 Another method is to target antigens directly to cells of the immune system through the use of MAb-Ags or other molecules.9 A variety of MAb-Ags have been targeted to antigen presenting cells via major histocompatibility complex (MHC) class II MAbs,10 to B cells via surface IgD11 or immunoglobulin Fc receptors12,13 and to dendritic cells (DC) via MAb-peptides Ags.14,15 In general, these MAb-Ags produce enhanced antibody responses. However, most studies of serum antibody responses examined secondary responses in which high titer affinity-matured IgG is produced. The utility of these approaches for facilitating the production of MAbs has not previously been investigated.

The immunogenicity of a vaccine candidate antigen is the ability to engender antibodies that recognize the corresponding native epitopes. Ideally, a subunit vaccine is comprised of an antigen that is not only immunogenic but also engenders protective antibodies. Research on synthetic peptide vaccines originated from early observations of immune responses to chemically synthesized linear peptides corresponding to protective

1The Skaggs Institute for Chemical Biology and the Department of Molecular Biology, The Scripps Research Institute, BCC-515, 10550 North Torrey Pines Road, La Jolla, CA 92037.
2Centro de Investigacion Cientifica y de Educacion Superior de Ensenada. Departamento de Acuacultura, Biotecnologia Marina, Tijuana, Mexico.
*These authors contributed equally to this work.
epitopes of polio and foot and mouth disease viruses.\textsuperscript{(16,17)} In these cases the protective peptides were identified by epitope mapping with neutralizing serum or MAbs. Unfortunately, the peptide vaccine field in general suffers from a serious general drawback—low immunogenicity. While peptide-carrier combinations can be antigenic (stimulate anti-peptide antibodies) these antibodies tend to recognize the native epitopes poorly. Low immunogenicity has been attributed to many factors including conformational flexibility of the native antigen, and the low amount of native structural information portrayed in small synthetic peptides. Alternatively, studies with a highly characterized model peptide epitope in various conformational constraints reveals that the MAb on-rates, rather than conformation, correlates with affinity of the MAbs raised to these structures.\textsuperscript{(18)} While this may be a characteristic limited to MAb-peptide binding studies, even these model antigenic determinants required co-delivery with adjuvants and multiple boosters in order to produce high titer IgG responses. Because concentration may be more important than absolute affinity for antibody-mediated protection,\textsuperscript{(19)} the ability of a vaccine to rapidly engender a high titer, secondary-like, response may be more important than the absolute affinity of the antibody produced.

From an applied perspective, the generation of rapid high titer IgG responses by \textit{in vivo} DC targeting in mice should facilitate MAb production. DC are sentinels of the immune system and are capable of stimulating powerful antigen-specific immune responses. DC have become the focus of many new vaccine strategies. Importantly, targeting of MAb-Ags to DC stimulates primary antibody responses by B cells.\textsuperscript{(20,21)} Similar strategies may induce protective primary antibody responses against infectious or toxic threats if given just before or soon after exposure.\textsuperscript{(15)} The isolation of monoclonal antigen specific antibodies from MAb-Ag immunized animals would also serve as an unequivocal confirmation of the MAb-Ag targeting technique. In this paper we sought to extend the findings of Wang et al.\textsuperscript{(15)} by evaluating DC targeting of synthetic peptide MAb-Ags as an \textit{in vivo} method for the rapid production of MAbs.

\textbf{MATERIALS AND METHODS}

\textbf{Targeting antibody–antigen preparation}

N418 hybridoma [American Type Culture Collection (ATCC)] was grown in the Integra CELLine CL1000 with BD-Cell-MAb media. The CL1000 was inoculated with $1 \times 10^8$ cells in 15 mL of BD-Cell, 20% FCS. Purified antibody was dialyzed twice against PBS and stored frozen at $-80^\circ$C until used further.

Synthetic peptides were commercially synthesized and HPLC purified (United Biochemical Research Inc., Seattle, WA). Two synthetic peptides were used; one, termed the C-terminal peptide (H\textsubscript{2}N-CSRDEEQFLSPAPATPNPPPA—COOH) corresponds to the unique C-terminal residues of the DC-SIGN protein;\textsuperscript{(22)} and the second is called the MID-peptide (H\textsubscript{2}N-CPEKSKLQEIQYETLQKLAAVGEL—COOH) and is a consensus peptide corresponding to a domain repeated eight times between the membrane and the C-terminal lectin and was previously to raise antisera to DC-SIGN.\textsuperscript{(23)} This sequence is also common to the recently discovered DC-SIGNR protein.\textsuperscript{(24)} The peptides were conjugated to the purified N418 IgG MAb pretreated with SMCC linker (Pierce, Rockford, IL) through the designed free N-terminal cysteine residue. MALDI mass-spectroscopy was used to evaluate linkage of the synthetic peptides to the N418 molecule.

Mice were immunized with 2 $\mu$g of these conjugates, half to intraperitoneal (i.p.) and to intradermal (i.d.) locations simultaneously. Following 7 days the animals were bled from the tail vein, the serum was treated at 37°C for 1 h, then 4°C overnight, and clots removed. Sera was serially diluted in 96-well V-bottom plates using 2%-bovine serum albumin (BSA) phosphate-buffered saline (PBS) as diluents. Standard enzyme-linked immunosorbent assay (ELISA) was performed using the same peptides linked to BSA at 100 ng/well. ELISA assays were stained with a commercial goat anti-mouse IgG—horse-radish peroxidase (HRP) conjugated antibody (Southern Biotechnology Associates, Birmingham, AL) at 1/1000 dilution in 2% BSA-PBS. The ELISA was developed with standard 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) substrate in citrate buffer (pH 4.4) and 0.2% H\textsubscript{2}O\textsubscript{2}. The plates were read at 405 nm after 1 h. The mice display reciprocal reactivity confirming peptide specific immune responses and not reactivity to the common SMCC-linker group used for both BSA and IgG linkage of peptides. The serum dilution used in all ELISA assays was 1/250 in 2% BSA-PBS, unless otherwise noted.

\textbf{Recombinant DC-SIGN protein construction and purification (pmalA’/pgexA’)}

Recombinant fragment of the human DC-SIGN protein containing the external repeat domains from residues 62 to 260 in the mature protein (termed A’ fragment) was cloned. The domain is found between (and excluding) the putative transmembrane domain and the C-terminal C-type lectin. Total RNA was harvested with an RNEasy kit (Qiagen, Valencia, CA) on >2.5 $\times$ 10\textsuperscript{6} human primary DC (BioWhittaker, Walkersville, MD). Complementary DNA was produced using the Superscript RT system (Invitrogen, Carlsbad, CA) and the cDNA was amplified using specific primers and high-fidelity polymerase (Invitrogen). Electrocompetent XL-1 blue (Stratagene, La Jolla, CA) \textit{E. coli} colonies were screened for inserts using polymerase chain reaction (PCR) and the sequence of the insert confirmed by cycle sequencing. The DC-SIGN A’ fragment was subcloned using the same conditions into the Sfi1 precut vector pGex4T (Amersham Biosciences, Piscataway, NJ) to contain Sfi1 sites. Colonies determined to be insert positive using vector specific primers and were sequenced to confirm identity. Clones with correct DC-SIGN fragment sequences in pMalp and pGex4T were expressed as fusion proteins (pMal-A’ and pGex-A’, respectively). Both pMal-A’ and pGex4T-A’ were expressed in XL-1 blue \textit{E. coli}. Protein was purified from sonicated cell lysates using amyllose resin columns (New England Biolabs, Beverly, MA). For pGex4T-A’, expression was carried out in two 500-mL super broth cultures. Each culture was induced for 6 h with isopropyl-beta-D-thiogalactopyranoside (IPTG) (0.3 mM) when the optical density (OD) was between 0.5 and 2.0. Purification from sonicated cell lysates was achieved using glutathione sepharose-4B slurry (Amersham Biosciences). The eluted samples were concentrated and
brought into PBS using Centriprep –10 sec (10,000 MW cut-off) (Millipore, San Clemente, CA). Protein size was confirmed by Coomassie staining of protein gels run against prestained MW marker (BioWhittaker) upon premade 15% sodium dodecyl sulfate (SDS)-polyacrylamide gels (PAGE) (Biorad, San Diego, CA).

**Fusion reagents and hybridoma production**

Splenocytes were harvested 10 days postimmunization, and fused to the P3X63Ag8.653 myeloma in log phase according essentially to the protocol of Lane. Briefly, the PEG1500 (Roche Molecular Biochemicals, Mannheim, Germany) was added drop-wise over 1 min while gently tapping the tube containing the myeloma-splenocyte pellet on the flow hood surface. The cells were swirled for an additional minute. The polyethylene glycol (PEG) was diluted by addition of 8 mL of serum-free RPMI media (RPMI-0; BioWhittaker) over 1 min. The cells were washed twice in RPMI-0, and resuspended in incomplete RPMI with 10% fetal calf serum, FCS, and 4% hybridoma cloning factor (IGEN International Inc., Gaithersburg, MD) and hypoxanthine, aminopterin, and thymidine (HAT) additive (Invitrogen). Cells were plated out to 10 96-well tissue culture plates (BD Biosciences; San Diego, CA) and cultured at 37°C and 5% CO₂ overlay. Tissue culture supernatants were assayed for binding to peptide-bovine serum albumin (BSA) conjugates in ELISA when foci were visible to the eye. The Costar 3690 96-well ELISA plates (Corning, Acton, MA) were coated with respective peptide-BSA conjugates (100 ng/well) in PBS overnight at 4°C and then blocked with 4% BSA, 1% Albumin in PBS, for 2 h at 37°C. Supernatant (100 μL/well) was incubated neat for 1 h at 37°C. The ELISA plates were washed ten times with dH₂O and patted dry on a paper towel. A pan-goat anti-mouse IgG-HRP antibody (Southern Biotechnology Associates; Birmingham, AL) was used at 1:2000 in 2% BSA in PBS for 45 min at 37°C and washed as above. Binding was detected with ABTS. The OD was read at 405nm at 1 h of development, or as indicated. Fusion efficiency was monitored in parallel by testing the supernatants for IgG production using enzyme immunoassay (EIA) plates coated with 200 ng/well of unconjugated, pooled, goat anti-mouse kappa and lambda light-chain antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) for IgG capture. Wells corresponding to positive signals for a single peptide-BSA conjugate were retested and subcloned at limiting dilution twice. MAbs were isotype at the 6 well-stage with a mouse-HRP clonotyping kit (Southern Biotechnology Associates) with optimized working dilutions and developed as above.

**Flow cytometry on normal human cells**

Normal human dendritic cells (NHDC, blood-derived CD14⁻) and human umbilical-vein endothelial cells (HUVEC) were purchased from BioWhittaker. Culturing of NHDC was performed in LGM-3 growth media (BioWhittaker) complete with albumin, insulin, transferrin, gentamicin, GM-CSF (500 U/mL), IL-4 (1000 U/mL), and 5% of human plasma. HUVEC were maintained in EGM growth media (BioWhittaker) complete with hEGF, hydrocortisone, insulin, gentamycin, amphotericin-B, and 2% fetal bovine serum (FBS). The EGM media was supplemented with Bovine Brain Extract before use.

(BioWhittaker). After 4 days of culture, NHDC and HUVEC were washed once with N₂-hydroxyethylpiperazine-N₂ethane-sulphonic acid (HEPES) Buffered Saline Solution (HBSS; BioWhittaker) and harvested by mild trypsinization with 0.025% trypsin, 0.01% ethylene-diaminetetraacetic acid (EDTA) in HBSS (BioWhittaker). NHDC found in suspension (non-adherent) were also collected by extensive washing with PBS, and stained separately from the remaining adherent NHDC. NHDC were washed once with PBS, resuspended in 1 mL of LGM-3, and incubated for 1 h at 37°C in the presence of 1 mg/mL of human IgG to prevent binding through Fc portion of the Abs. Cells were washed once with PBS and resuspended at 10⁶ cells/mL of FACS buffer (1% BSA, 0.03% NaN₃, 25 mM HEPES in PBS, pH 7.4 sterile filtered). Aliquots of 100 μL containing about 10⁶ cells were distributed into wells of V-bottom serocluster plates (BD Biosciences). One hundred microliters of purified from hybridoma cultures MAb (5 μg/mL) were then added to the cells and incubated for 30 min at room temperature (RT). Cells were washed once (spun down at 1800 rpm for 2–3 min) with 200 μL of FACS buffer and incubated with 100 μL of fluorescein isothiocyanate (FITC)-conjugated affinity-purified donkey anti-mouse IgG (H+L) antibody (Jackson ImmunoResearch Laboratories), diluted to 1:100 in FACS buffer for 30 min at RT. Cells were washed twice as above, resuspended in 200 μL of FACS buffer, and transferred to FACS-tube for analysis in a FACS scan flow cytometer with CellQuest software (Becton Dickinson, San Jose, CA).

**RESULTS**

**Conjugation of synthetic peptides to N418 (anti-CD11c)**

The purified hamster IgG MAb N418 was used as the carrier moiety and coupled to either the MID- or C-terminal peptides. Coupling was mediated using commercial reagents and the terminal Cys residues instituted by design. Coupling efficiency was evaluated by a shift in molecular weight of the IgG molecules on SDS-PAGE gels (data not shown) and confirmed using mass spectroscopy. The average molecular weight of the IgG with coupling groups was about 159,078 kDa as predicted, and the size shifted to 175,931 kDa with the MID-peptides groups attached (Fig. 1). The MID peptide is 2200 kDa therefore we estimate that the average valence of peptide attachment is approximately 7 peptides per N418 hamster IgG molecule.

**Serological responses to MAb-Ag conjugates**

Groups of mice were immunized with MAb-peptide Ag conjugates and tested for homologous and heterologous serum IgG reactivity on Day 7. The specific serum IgG was tested on the same synthetic peptides conjugated to BSA as antigen. Groups of mice (n = 4–6) were immunized with N418 conjugated with the C-terminal peptide, or the MID-peptide, or C-terminal peptide and N418 unconjugated. A fourth group of mice was immunized with N418 MAb but with only half of the molecules conjugated to the C-terminal peptide. Mice immunized with the C-terminal peptide MAb-Ag have a serum IgG end-point titer of 1/6000 for the homologous peptide-BSA conjugate and no reactivity with the MID-peptide BSA conjugate. Conversely,
mice immunized with the MID-peptide MAb-Ag have a serum IgG end point titer of 1/1200 for the homologous peptide-BSA conjugate, and no reactivity for the C-terminal peptide BSA conjugate (data not shown). The clear reciprocal reactivity of the serum indicated that the rapid IgG response is specific for the peptide moieties, and not the common linker groups (Fig. 2). Mice co-immunized with naked N418 and C-terminal peptide conjugated N418 show a reduced serum IgG reactivity for the homologous C-terminal peptide-BSA conjugate (Fig. 2). This may reflect a dose-dependent or competitive effect for CD11c on the DC. Co-immunization of mice with N418 MAb and free C-terminal peptide does not produce measurable serum IgG to either peptide-BSA conjugates by Day 7. Similarly, mice immunized with free MID-peptide and N418 do not produce measurable serum IgG by Day 7 to either peptide-BSA conjugate. These data show that conjugation of peptides to the anti-CD11c antibody are required to elicit rapid IgG responses.

FIG. 1. Overlay of the MAb–Ag conjugates analyzed in mass spectroscopy. The upper line represents the hamster MAb N418 conjugated to the MID-peptide. The lower line represents the same purified IgG with coupling reagent but without peptide attachment.

FIG. 2. Serum IgG reactivity for peptides. Serum was collected 7 days after a single immunization of the MAb-Ag conjugates without adjuvant. The analysis shown was done at a 1/250 dilution of serum. White bars represent C-terminal peptide IgG reactivity; Black bars represent MID-peptide IgG reactivity. Each column represents the averaged value of the indicated number of mice. The standard errors are shown.
MAbs from fusion at Day 10

The splenocytes from a single mouse immunized with N418-MID-peptide and N418-C-terminal peptide were harvested on Day 10. Three hybridomas were identified as MID-peptide reactive from 250 IgG-producing wells and were selected and grown for further studies. All three of these MAbs are of the IgG1/kappa isotypes. This is consistent with the observations of Wang et al.\(^{15}\) as IgG\(_1\) was the predominant IgG class observed in the rapid responses to their model antigens. These MAbs react with the MID-peptide BSA conjugate specifically and not with the C-terminal peptide nor with the common linker group (Fig. 3). Fusion efficiency was far lower for the C-terminal peptide immunized mouse, as there were no C-terminal peptide specific clones identified from the 80 IgG producing wells. This is despite the fact that the serological response of the C-terminal peptide immunized mice were 5 times higher, and the splenic inflammation was far greater for the C-terminal immunized mouse compared with the MID-peptide immunized mouse. This shows that fusion efficiency still cannot always be predicted based upon phenotypic markers.

Specificity of anti-DC-SIGN MAbs

The MID-peptide MAbs were tested for reactivity with recombinant DC-SIGN fragments. Two recombinant DC-SIGN fragments spanning the repeat-domain from which the MID-peptide corresponds, were tested for binding in ELISA. One of the clones, Novik2, reacted with recombinant DC-SIGN proteins (Fig. 3). Novik2 does not react with irrelevant pGex and pMaL fusion proteins and is the only MAb to react with whole DC-SIGN expressed in cell lysates in western blot (data not shown). Thus, our recombinant DC-SIGN proteins are antigenic for only one of three linear peptide epitope specific MAbs. The binding of Novik2 to both pMaL-A’ and pGex-A’ was specifically inhibited by soluble MID-peptide, but not by the C-terminal-peptide (Figs. 4A and B, respectively). This shows that the binding of Novik2 was specific for the MID-peptide epitope in the recombinant DC-SIGN A’ fragments.

**DC-SIGN expression on NHDC**

Finally, we tested the two MAbs, Novik2 and 3, for reactivity with native DC-SIGN on NHDC in flow cytometry. We found that two MID-peptide specific MAbs reacted with a reproducibly small percentage (8–11%) of nonadherent NHDC subpopulations, which had high levels of DC-SIGN expression, compared with isotype matched control (Figs. 5E and F). Blood-derived DC are known in the literature to express DC-SIGN in between 4–14% of their population\(^{(26)}\). Thus, our anti-DC-SIGN MAbs that bind fit precisely into this range. In contrast, only Novik2 MAb was able to detect DC-SIGN, which was weakly expressed by adherent NHDC subpopulation (Figs. 5C and D; large peaks shifts to right for Novik2). Novik3 was unable to detect the lower level of DC-SIGN expression. The small bright fluorescent peak of cells are most likely due to remaining loosely adherent DC, which contaminate the adherent cell population after our imperfect separation of DC subpopulations.

**FIG. 3.** Reactivity of MID-peptide MAbs on recombinant human DC-SIGN A’ fragment and the corresponding DC-SIGN peptides. Binding of three MID-peptide-specific MAbs identified by hybridoma technique is shown. The average of three different assays is shown.

**FIG. 4.** Peptide inhibition of MAb binding. Novik2 MAb binding on pMaL-A’ fragment (A) and pGex-A’ fragment (B). The black squares represent MAb binding in the presence of C-terminal peptide; Black circles represent binding in the presence of MID-peptide; Open circle represents binding in the absence of peptide inhibitors. The average of three different assays is shown.

*IN VIVO DELIVERY OF PEPTIDE EPITOPES*
based upon gross phenotype of being adherent or nonadherent NHDC in tissue culture. HUVEC were uniformly negative for DC-SIGN (Figs. 5A and B). This shows that the synthetic peptides are immunogenic for the native DC-SIGN protein and suggests that the peptide epitopes in the A′ region are more accessible on DC cells than in recombinant fusion proteins.

Overall, these findings indicate that the MAb-Ag peptide DC targeting strategy is useful for the rapid generation of MAb to predetermined epitopes.

**DISCUSSION**

The DC-SIGN-specific MAb described herein are the first set of MAbs produced using the immunotargeting MAb-Ag technique. They are specific to a peptide-epitope from DC-

SIGN. DC-SIGN is a type II membrane protein with a C-terminal lectin-binding domain, and is involved in DC interaction with other immune system cells. DC-SIGN was initially cloned from the cDNA of human placenta and it was subsequently shown to be highly expressed on DC and to bind ICAM-3. Recently, a closely related homolog to DC-SIGN, termed DC-SIGNR was described, that exhibits 77% amino acid identity with DC-SIGN. The DC-SIGNR expression was documented on endothelial cells in human placenta, lymph node sinuses, and hepatic sinusoids, but not on peripheral blood-derived DC. Using the MAb-Ag technique with DC-SIGN peptides borne on a DC targeting MAb-Ags, we obtained specific hybridomas in 8 weeks. This marked reduction in the 2–5 months normally needed to immunize and boost an animal prior to the fusion and subcloning process will directly translate into savings of time and money.
In the present study, we demonstrate that the N418 MAb-Ag system is useful for the rapid generation of antigen specific MAbs. Moreover they show that the IgG1 class of antibodies is generated within days of a single-step immunization by targeting CD11c+ cells, including DC, of the mouse. This reduces the amount of time needed to house and immunize animals compared to normal priming and boosting strategies. Indeed a single immunization of just 2 μg of antigen allowed us to produce peptide specific, recombinant protein reactive, and native cell expressed protein reactive MAb in just 8 weeks. The time required for the in vitro cloning can be reduced further through the use of direct picking of individual foci from in soft-agar\(^{(29)}\) the conditions for which have been greatly redefined (R.P. Beecroft and T.W. Pearson, University of Victoria, Canada, personal communication). Thus, the production of MAbs via hybridoma fusion from immune mice has a secure future in antibody discovery in particular in light of new technologies such as the generation xenomice expressing nonrodent V-genes.\(^{(30-32)}\) The rapidity of the IgG response is intriguing from an immunological standpoint. A rapid serum IgG response is engendered to the synthetic peptide conjugates by Day 7, the magnitude of these IgG responses is more modest with respect to those seen by Wang et al.\(^{(15)}\) Recently, Wang et al.\(^{(15)}\) showed that targeting model antigens to the mainly DC borne antigen-CD11c via a hamster anti-mouse CD11c MAb N418\(^{(33)}\) enhanced the serum IgG antibody levels in the primary response. A single low-dose immunization of MAb-Ag, in this case a hapten or goat anti-hamster antibody, produced high titer IgG antibody in only 7 days without adjuvant.\(^{(15)}\) The mice had serum IgG titers as high as 1:100,000 to the goat moiety as early as Day 7. Remarkably, this was in naive mice without the use of any classical adjuvants and an irrelevant hamster MAb conjugated to the same antigen produced little measurable serum IgG until Day 20. Clearly, this type of response will vary depending on individual antigens and on the parameters of this type of response remain to be determined. We thereby extend these results by demonstrating the use of in vivo DC targeting to accelerate MAb discovery. The predominant IgG class recovered by Wang et al.\(^{(15)}\) was IgG1, which is consistent with the isotype of the MAbs we recovered in our fusion.

The molecular basis for this enhanced response remains to be determined. Similar rapid and potent IgG responses are seen in some viral antigens. Viral particle with repeating antigenic structures on cytopathic viruses elicit rapid antibody responses.\(^{(34)}\) While the basis for this rapid response is also not clear, some general findings are relevant. Sequencing of many VSV MAbs from early and late in the response showed that multiple V-region genes are recruited early on in the response.\(^{(35)}\) Remarkably, the MAbs selected early to VSV on Day 7 had the same high affinity as those from late in the immune response. It is likely that the repeat structures of viruses such as VSV may bind directly to DC receptors triggering a rapid B-cell response and generation of antibody. Affinity studies on DC targeted MAb-Ags at different time points will test the generality of this effect. The influence of DC in the recruitment of B-cell clonotypes and studies of V-gene usage and mutation may clarify the T-cell dependence of B cells producing these rapid responses.\(^{(36,37)}\) For example, DC are capable of eliciting B-cell responses directly to live cytopathic virus in a CD40 independent\(^{(38,39)}\) and T-cell independent manner\(^{(38)}\) they tend to concomitantly stimulate T-cell help. The structure of the antigen is apparently important as T-cell-independent antiviral antibody responses were seen in the VSV glycoprotein when expressed as a repetitive antigen but not as an irregular cell surface protein.\(^{(19)}\) Indeed, the more rigidly arrayed the VSV glycoprotein, the less B-cell primary and secondary responses are T-cell dependent.\(^{(34)}\) Evidence suggests that the DC are intimately involved in the rapidly protective antibody response to live but not UV inactivated VSV virus.\(^{(39)}\) We speculate that the targeting of MAb-conjugated antigens onto the surface of murine DC cells may similarly serve to localize Ag into repeat-like structures on the surface of the murine DC. If this strategy translates to protective epitopes in other systems it would be valuable as a biomedical countermeasure in the event of an intentional release of a pathogen or its products for postexposure immunization, or for a rapid boost to immunity of individuals who may be exposed to a pathogen in short order.\(^{(15)}\) Currently we do not know the longevity or the protective capacity of this type of antibody response.

These data confirm the general utility of the immunotargeting approach\(^{(9)}\) for producing rapid antibody responses in mice. The enhanced immunogenicity of synthetic peptides delivered as MAb-Ags to DC in vivo may overcome past problems of limited immunogenicity that subdued the initial promise of peptide vaccines.\(^{(16,17,40)}\) In support, synthetic peptide vaccines delivered to the mucosa of the nose and to the skin (areas rich in DC cells) have efficient systemic and mucosal immune responses.\(^{(41)}\) We would also predict that the MAb-Ag technique would work as a mucosal immunization strategy in mice as these tissues are rich in DC. Clearly synthetic peptide-based vaccines will not be protective in all cases;\(^{(42)}\) however, the in vivo DC targeting strategy can work for whole proteins.\(^{(15)}\) Clearly, further studies are warranted to determine if such rapid IgG responses can be engendered against whole microorganisms or toxins, and if these antibodies provide in vivo protection against challenge with the live organisms. It will also be interesting to see if the same MAb-Ag strategy can translate to larger animals and humans. In humans, we speculate that DC-SIGN targeting antibodies, like the ones prepared here, might serve as appropriate DC-targeting MAbs for the MAb-Ag approach described here. This technique uses extremely well-defined and characterized subunit immunogens. In this respect, the MAb-Ag method is particularly well suited to accommodate antibody discovery, via hybridoma or phage display, in the rapid field of genomics. The pace at which new target proteins are being identified has greatly exceeded the ability of immunologists to produce quality MAb reagents. Clearly, the development of techniques that reduce the amount of time cost and effort are critical for the efficient production of MAbs and the development of new vaccines based on such proteomic experiments. In summary, by using in vivo DC targeting with MAb-Ags, we have demonstrated here the rapid generation of MAbs specific for a peptide epitope of human DC-SIGN.

ACKNOWLEDGMENTS

The authors thank Mike Kubitz for excellent technical assistance on the hybridoma production. Our thanks also to the Scripps Research Institute Department of Animal Services and Department of Mass Spectrometry. JDB is supported by a Cana-
 REFERENCES

35. Kalinke U, Buccher EM, Ernst B, Oxenius A, Roost H, Geley S,

36. Andria M, Levy S, and Benjamini E: Diverse VH and VL genes are used to produce antibodies against a defined protein epitope. *J Immunol* 1990;144:2614-2619.

37. Manser T: Mitogen driven B cell proliferation and differentiation are not accompanied by hypermutation of immunoglobulin variable region genes. *J Immunol* 1987;139:234-238.


Address reprint requests to:
Carlos F. Barbas, III, Ph.D.
The Skaggs Institute and Department of Molecular Biology
The Scripps Research Institute, BCC-526
10550 North Torrey Pines Road
La Jolla, CA 92037

E-mail: carlos@scripps.edu

Received for publication September 13, 2002. Accepted for publication November 20, 2002.