PB10 is a murine monoclonal antibody against an immunodominant epitope on ricin toxin’s enzymatic subunit. Here, we characterize a fully humanized version of PB10 IgG1 (hPB10) and demonstrate that it has potent in vitro and in vivo toxin-neutralizing activities. We also report the minimum serum concentrations of hPB10 required to protect mice against 10 times the 50% lethal dose of ricin when delivered by injection and inhalation.

PB10 is a murine monoclonal antibody (MAb; mPB10) with potent toxin-neutralizing activities (TNA) in vitro and in vivo (8). PB10 recognizes a linear epitope within an immunodominant region on RTA referred to as cluster I (9, 10) and is proposed to neutralize ricin by interfering with toxin transport to the TGN (11). We recently characterized a chimeric version of PB10 (cPB10) in which the murine V\(_1\) and V\(_{H1}\) domains of mPB10 were genetically fused to human IgG1 and \(\kappa\) constant regions, respectively, and then we expressed this construct by using a Nicotiana benthamiana-based rapid antibody-manufacturing platform (RAMP) (12). cPB10 proved to be the most effective of the four different chimeric MAbS that were tested for neutralizing activity in in vitro cell-based cytotoxicity assays and also in passive protection studies in mouse models of systemic and aerosol toxin challenge. cPB10 was also able to rescue mice from the effects of ricin when administered up to 3 to 4 h after toxin challenge. Finally, cPB10 retained full ricin toxin-neutralizing activities in vitro and in vivo when present in a tripartite anti-category B toxin (CatB) cocktail with chimeric IgG1s against staphylococcal enterotoxin type B (SEB) and Clostridium perfringens epsilon toxin (ETX) (13).

In the interest of developing a better product for human use, we investigated a fully humanized version of PB10 (hPB10). The murine V\(_1\) and V\(_{H1}\) domains of mPB10 were humanized by performing multiple alignments against the IMGT human V gene database. The native murine framework residues were selectively replaced with human framework residues, being mindful of potential contact amino acids that can span framework (FR) and complementarity determining region (CDR) junctions (14). More specifically, the murine variable region of PB10 was compared to human germ line V genes by using IgBLAST (15). PB10 framework domains showing less than 70% identity to the human germ line were also subjected to comparative analysis via IgBLAST. Single amino acid mutations were introduced into the PB10 V\(_{H1}\) and FR regions, based on the frequency with which the substitution was found in human germ line genes. FR/CDR junctions were also inspected but rarely altered, due to their potential to act as contact residues (14). The final complete sequence (variable and constant) of hPB10 was deemed to be >90% human based on this analysis. hPB10 was expressed using the N. benthamiana RAMP and subjected to traditional affinity chromatography purification as described previously (12). The yield of hPB10 was 200 mg/kg of leaf tissue, with a purity of >97% (data not shown).

We first characterized hPB10 for in vitro and in vivo toxin-binding and -neutralizing activities, alone or as part of a cocktail of MAbS (hCatB) targeting the Category B Select Agent toxins ETX, ricin, and SEB. hCatB consists of an equimolar combination of hPB10 with humanized versions of anti-SEB (hu19F1) and anti-
ETX (hu4D7) MAbs (13). Enzyme-linked immunosorbent assay (ELISA) and toxin-neutralizing assay protocols have been described previously (8, 13, 16).

By ELISA, hPB10 bound ricin holotoxin and RTA, and based on RTA pepscan analysis, hPB10 was specific for a peptide spanning residues 91 to 108 (Y91FFHPDNQEDAEAITHLF108) (Fig. 1), thereby demonstrating that humanization of the PB10 VL and VH domains did not negatively impact epitope specificity or affinity. When assessed for in vitro TNA using Vero cells, hPB10 and mPB10 had 50% inhibitory concentrations (IC50s) between 0.015 and 0.03 ng/ml (Fig. 1). The IC50 of mPB10 reported here is exactly what was reported previously (12). Reactivity profiles, as well as 50% effective concentrations (EC50s) and IC50s, were unchanged when hPB10 was mixed with equimolar amounts of anti-SEB (h19F1) and anti-ETX (h4D7) MAbs (data not shown).

We next determined the degree to which hPB10 was able to passively protect mice from systemic and mucosal ricin challenge, using protocols described previously (13). Female BALB/c mice, 6

| TABLE 1 hPB10 serum concentrations associated with protection against systemic and mucosal ricin challenge<sup>c</sup> |
|-----------------------------------------------|
| **Intraperitoneal challenge** |
| Initial i.p. dose (µg)<sup>a</sup> | Serum concn (µg/ml)<sup>b</sup> | Survival |
| 5 | 1.1 | 5/5 |
| 2.5 | 0.8 | 5/5 |
| 1.25 | 0.4 | 5/5 |
| 0.625 | 0.2 | 5/5 |
| **Intranasal challenge** |
| Initial i.p. dose (µg)<sup>a</sup> | Serum concn (µg/ml)<sup>b</sup> | Survival |
| 40 | 18.82 | 6/6 |
| 30 | 13.09 | 2/6 |
| 20 | 9.53 | 0/6 |
| 10 | 4.65 | 0/6 |

<sup>a</sup> hPB10 was administered to mice by the i.p. route at the indicated doses.

<sup>b</sup> Average serum hPB10 concentrations in blood taken 2 h prior to ricin challenge are reported.

<sup>c</sup> Values in boldface indicate the minimum serum hPB10 concentration associated with protection against ricin challenge.
FIG 2  Passive protection associated with hPB10 and the hCatB cocktail. hPB10 administered at the indicated doses (A to D) or hCatB cocktail (equivalent of 50 μg of hPB10) (E and F) was administered to mice by i.p. injection, and 24 h later mice were challenged with ricin by i.p. injection (A and B) or intranasal administration (C to F). Survival was monitored for 7 days. (B, D, and F) hPB10 levels determined from serum taken from mice 2 h prior to ricin challenge. (G) Mice were challenged with ricin by the intranasal route and then given 100 μg of hPB10 in the form of the hCatB cocktail at the indicated time points (i.e., 0, 2, 4, 6, 7 h). Survival was monitored for 10 days.
to 8 weeks of age, were purchased from Taconic Labs (Hudson, NY) and housed at the Wadsworth Center, New York State Department of Health, under conventional, specific-pathogen-free conditions. All animal studies were conducted in strict compliance with protocols approved by the Wadsworth Center’s Institutional Animal Care and Use Committee (IACUC). hPB10 and hCatB were administered at a range of doses to groups of mice by intraperitoneal (i.p.) injection on day –1 (Table 1). On day 0, blood was collected via the lateral tail vein from the mice in order to estimate serum hPB10 concentrations at the time of challenge. Approximately 2 h after blood collection, the mice received the equivalent of 10 × LD₃₀ for ricin (~2 μg per mouse) delivered by the i.p. injection (systemic) or intranasal (mucosal) routes. Following ricin challenge, animals were monitored twice daily for 5 to 10 days for symptoms of ricin intoxication (e.g., hypoglycemia, weight loss). Animals were euthanized over the course of the study based on criteria established by the Wadsworth Center’s IACUC. As expected, hPB10 was able to passively protect mice from both systemic and mucosal ricin exposure, although the thresholds of serum hPB10 associated with immunity were different in each case. In the systemic challenge model, full protection from ricin toxin was observed in the groups of mice that received 5 or 2.5 μg hPB10 (Table 1; Fig. 2A). Mice that received 1.25 or 0.6 μg hPB10 succumbed to ricin intoxication within 5 days. Analysis of serum antibody levels just prior to challenge suggested that protection occurred when hPB10 levels were ≥0.8 μg/ml serum (Table 1; Fig. 2B). In the intranasal challenge model, in contrast, full protection was observed only in the group of mice that received 40 μg hPB10 (Table 1; Fig. 2C). No appreciable protection was observed in animals that received 10 μg or 20 μg hPB10, while only 2/6 mice that received 30 μg hPB10 survived the challenge. Analysis of serum antibody levels just prior to challenge indicated that protection was achieved when hPB10 levels were ≥18 μg/ml (Table 1; Fig. 2D). This finding is significant because it establishes for the first time that >8 times more circulating ricin-specific antibody (i.e., hPB10) is required to protect the mice against intranasal challenge than against systemic challenge, even though the LD₃₀ values are the same between the routes of exposure. Presumably, the higher serum IgG levels are required to achieve transudation of hPB10 from the serum to the lung compartment in sufficient amounts so as to neutralize ricin before it gains entry into the mucosa. The intranasal challenge studies were repeated using the hCatB cocktail. Groups of mice received 50 μg of hPB10 or the equivalent of 50 μg of hPB10 in the form of the hCatB cocktail (Fig. 2E and F). The animals were challenged 1 day later and monitored for ricin intoxication. Analysis of serum just prior to challenge indicated that hPB10 levels were ~18 μg/ml in mice that had received hPB10 and 28 μg/ml in mice that had received the hCatB cocktail (Fig. 2F). As expected, ricin control mice succumbed to ricin intoxication within 72 h, whereas protection was observed in mice treated with hPB10 or the hCatB cocktail. The observation that serum hPB10 levels were reproducibly higher in mice that received hPB10 in the context of the hCatB cocktail than in those that received hPB10 by itself may be explained by delayed clearance of hPB10 when administered to mice in the presence of equimolar amounts of anti-SEB (h19F1) and anti-ETX (h4D7) humanized MAbs. To assess the therapeutic potential of hPB10, groups of mice (n = 6) were challenged with ricin by the intranasal route, as described above, and then administered the equivalent of 100 μg hPB10 in the form of the hCatB cocktail at time zero or intervals thereafter (i.e., 2, 4, 6, and 7 h). While full protection was only observed when hCatB was administered at time zero, partial protection (60 to 80%) was attained when MAb treatment occurred as late as 7 h post-ricin challenge (Fig. 2G). These results are proof of principle that hPB10 has the potential to serve as a therapeutic, even in the context of a stringent mucosal challenge, in which control animals succumb to ricin intoxication within 48 h. Future studies will include testing hPB10 and the hCatB cocktail at higher doses, as well as examining the degree to which systemic hPB10 protects the lung mucosa from the effects of ricin toxin. In summary, we have successfully produced a fully humanized IgG1 derivative of PB10, a murine MAb first identified for its ability to neutralize ricin in vitro and in vivo (8). While hPB10 needs to be fully vetted in a nonhuman primate model, our preliminary results from mice suggest hPB10 has potential utility as both a prophylactic and therapeutic agent against lethal ricin exposure. Moving forward, it will be important to compare the potency of hPB10 to other humanized antiricin toxin antibodies, such as D9 (17). D9 and hPB10 have similar in vitro and in vivo toxin-neutralizing activities, thereby warranting side-by-side animal studies. Finally we conclude that hPB10’s ricin-neutralizing activity is not compromised when combined with two other humanized antitoxin MAbs, hu19F1 and h4D7, raising the prospect of a therapeutic tripartite antitoxin that could be administered in individuals suspected of being exposed to ricin, SEB, or ETX (13). 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