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IMMUNOMODULATION BY MICROBIAL RIBOSOMES

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Over the past twenty years, many authors have reported evidence of the immunoprotective capacity of ribosomes isolated from bacteria, fungi and parasites.

Since 1971 we have explored the protective capacity of ribosomes isolated from a large variety of microorganisms responsible for human and animal diseases. More recently, using monoclonal antibodies raised against ribosomes and then selected for their ability to confer passive immunity to mice, we have studied the mechanism of the protection induced by ribosomes. These studies, in parallel with the development of a technology for the large scale production of ribosomes, have allowed us to achieve a new regard for ribosomal vaccines for use in human.

The general concept of ribosomal vaccines in presented and examples of two such vaccines are described with data on the specific protection that they induce in mice against experimental infections with Klebsiella pneumoniae, Streptococcus pneumoniae, S. pyogenes *and* Haemophilus influenzae *for the first one, and against* Candida albicans *type A and type B for the second one.*

Because of their high immunogenicity and their innocuity these vaccines represent a decisive improvement over classical microbial vaccines.

The first experimental evidence of the immunoprotective capacity of bacterial ribossomes was reported by Youmans in 1965 (Youmans & Youmans, 1965), demonstrating that 1 μg of *Mycobacterium tuberculosis* ribosomes of the H37Ra strain protected mice. Youmans had also shown that highly purified ribosomes require Freund's Incomplete Adjuvant (FIA) to recover their full immunogenicity (Youmans & Youmans, 1967).

Several authors have since published data demonstrating that ribosomes from a large variety of bacteria, fungi, and parasites were able ato induce strong homologous and to a certain degree heterologous protection (Au & Eisenstein, 1981; Everhart et al., 1984; Gonggrijp et al., 1981).

At the Center of Immunology and Biology P. Fabre, we have, since 1971 been studying the immunoprotective capacity of ribosomes isolated from about 200 microorganisms responsible for human and animal diseases, confirming their potency as efficient and safe vaccines.

As FIA cannot be used in humans, we have also isolated from the membrane of a non-capsulated strain of *Klebsiella penumoniae*, a proteoglycan exibiting strong adjuvant properties toward the ribosomes and remarkable immunostimulant activities (Millet et al., 1987; Normier et al., 1985).

Mechanism of the protection induced by bacterial ribosomes

While the vaccinating capacity of ribosomes is well established the mechanism by which they induce protection and more especially the exact nature of their "Immunogenic Principle" has been more difficult to clarify (Pinel et al., 1985a, b; Robert et al., 1984).

For this purpose our approach has been to raise monoclonal antibodies (mAbs) against ribosomes, which were further selected according to their capacity to confer passive protection, in mice, against a homologous experimental infection. The mAbs with protective capacity were then used to identify corresponding epitopes on ribosomes.

The following graphs will show several examples of the direct protection induced in mice with ribosomes against homologous infections as well as the passive immunity conferred by corresponding mAbs.

The first graph gives data obtained with 2 groups of 20 BALB/c mice immunized with 2 subcutaneous (s.c.) injections of *Klebsiella pneumoniae* type I ribosomes, or PBS in the control group, at days 0 and 14. At day 21 all mice were challenged intraperitoneally (i.p.) with 1.5×10^3 living cells. The graph represents the mortality in both control and treated groups during 20 days post-infection. One can see that all control animals died within 2 days while 80% of the mice treated with ribosomes survived.

Fig. 2 presents an experiment in which mice were immunized (i.p.) with mAb D12G6 raised

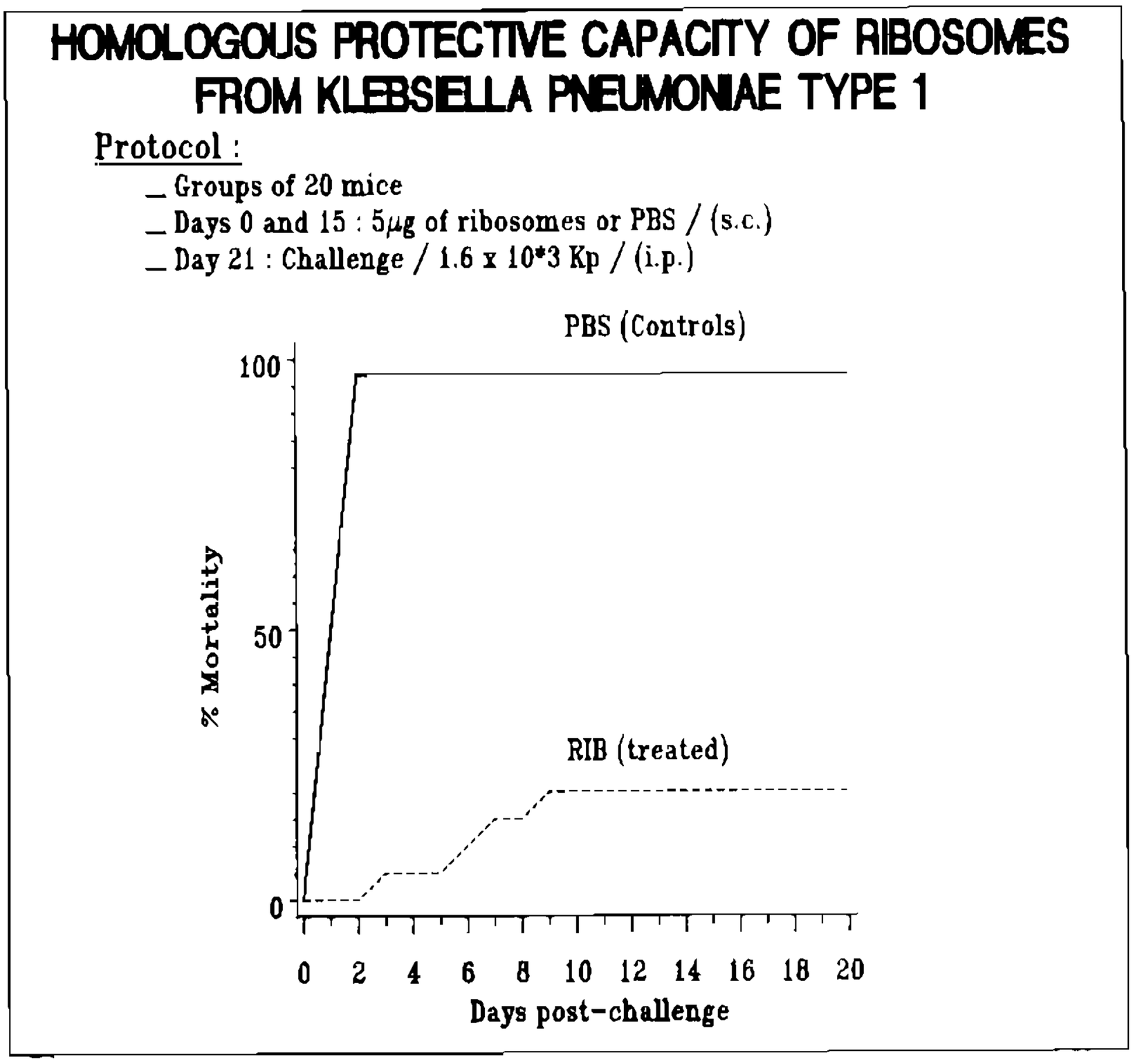

Fig. 1: Striking decrease in the mortality rate of Balb/c mice pre-treated with homologoud type I ribosome fraction, and then challenged with living *Klebsiella pneumoniae*.

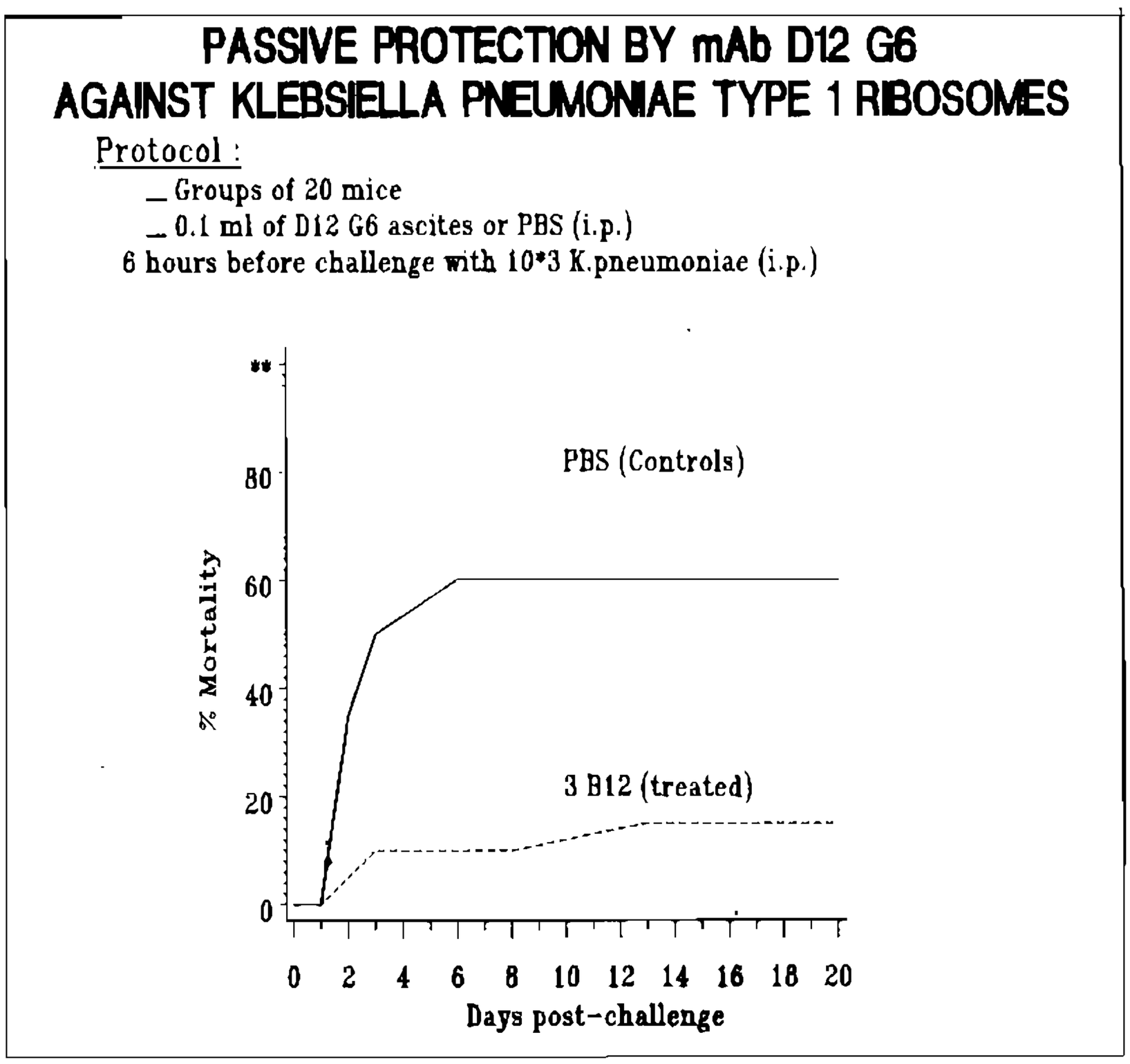

Fig. 2: Protective action of the anti-*Klebsiella pneumoniae* type I ribosome monoclonal antibody DIZ G6 on Balb/c mice infected with *K. pneumoniae*.

against *Klebsiella pneumoniae* ribosomes, 6 hours before (i.p.) infection with 10^3 living cells. Data presented on the graph clearly indicate that mAb D12G6 passively protects mice. Further studies using ELISA with purified antigens of *Klebsiella pneumoniae* have shown that mAb D12G6 is directed against the capsular polysaccharide (Ps-K) of this strain. Biochemical investigations on ribosomal subfractions have also allowed us to establish that cytoplasmic precursors of the PS-K are strongly linked to ribosomal-RNA (r-RNA) in trace amounts. These r-RNA-PS.K complexes after isolation from ribosomes are highly protective when adjuvated, while the purified PS-K injected alone or adjuvated failed to protect mice even at doses 50,000 times higher than that present in the protective dose of ribosomes.

Fig. 3 shows the protection of BALB/c mice immunized with *Streptococcus pneumoniae* type I ribosomes against a homologous lethal infection. Groups of 20 mice were immunized with 2 (s.c.) injections of 0.9 g of *Streptococcus pneumoniae* type I ribosomes at days 0 and 14. Controls received PBS alone. At day 21, 10^3 living cells were injected (i.p.). The mortality recorded during the 20 following days is represented on the graph. Only 5% of mice injected with ribosomes died from the lethal infection.

Fig. 4 gives the results of the passive protection conferred to mice by mAb 3B12 raised against *Streptococcus pneumoniae* tipe I ribosomes. Groups of 20 mice received intravenously (i.v.) mAb 3B12 or PBS for the control group 2 hours before (i.p.) infection with 10^2 living cells. The graph representing the mortality in each group indicates that 85% of the treated mice survived the challenge versus 5% only in the control group.

Additional studies with this mAb, recognizing a phosphorylcholine containing site of the pneumococcus membrane, have shown a significant degree of cross- protection with at least 8 different serotypes of *Streptococcus pneumoniae*.

Fig. 5 shows an interesting example of protection experiment using ribosomes isolated from two strains of *Streptococcus pyogenes* group A. The parent strain, non virulent for mice was rendered highly virulent after several passages on mice and reisolation. One can observe that only ribosomes isolated from the virulent strains were protective.

Additional studies have shown that only the virulent strain produced M-protein and that ribosomes isolated from this strains were linked to small amounts of M-protein (0.015% w/w). This was demonstrated by means of a monoclonal antibody raised against the M-protein purified from this strain (mAb C3G12).

Fig. 6 shows the passive protection of mice receiving (i.p.) 0.2 ml of C3G12 ascites 2 hours before challenge with 10^2 cells (i.p.) of the virulent *Streptococcus pyogenes*.

General concept of ribosomal vaccines

From the results obtained with these different models, and from biochemical studies not reported here we can say that the protection induced by ribosomes is related to their ability to induce protective antibodies against cell surface antigens.

Several groups in the world, like us, have shown that strong links exist between ribosomes and small amounts of membrane antigens (Ader & Arvison, 1984). Ribosomes strongly increase the immune response to these antigens at very low doses. This was observed even in the case of non immunogenic antigens such as *Klebsiella pneumoniae* polysaccharides.

Fig. 7 represents the general concept of ribosomal vaccines that we have developed, associating the specific immunity induced by ribosomes linked to protective epitopes, to the non-specific immunostimulating properties of a proteoglycan isolated from the membrane on a non-capsulated strain of *Klebsiella pneumoniae*.

Starting from this concept we have developed a technology for large scale production of ribosomes and also the membrane proteoglycan of *Klebsiella penumoniae* (M-PG kp) used as adjuvant.

Protective monoclonal antibodies against each ribosome are used as specific reagents for the control and the standardization of each batch of production.

Derived from this technology several ribosomal vaccines have been prepared for human as well as animal diseases.

One of these vaccines (D.53) which is now used in humans since more than ten years in respiratory infections under injectable, spray or tablet forms is composed of:

Ribosomes from:

- *Klebsiella pneumoniae* . . . . . 3.5 parts
- *Streptococcus pneumoniae* . . . 3.0 parts
- *Streptococcus pyogenes* . . . . . 3.0 parts
- *Haemophilus influenzae* . . . . . 0.5 parts

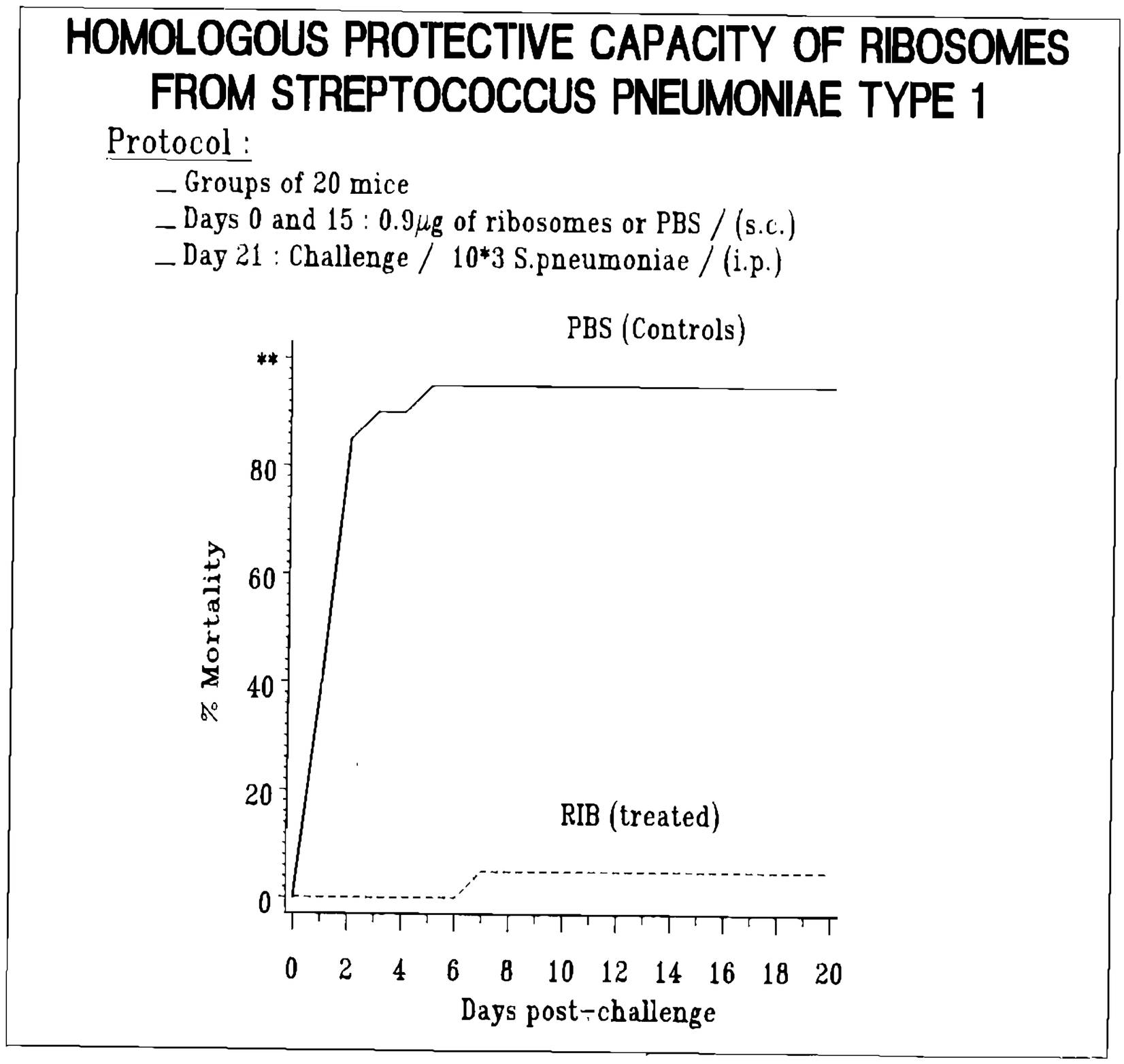

Fig. 3: Decrease in the mortality rate of Balb/c mice pre-treated with *Streptococcus pneumoniae* type I ribosomes and then challenged with a homologous letal infection.

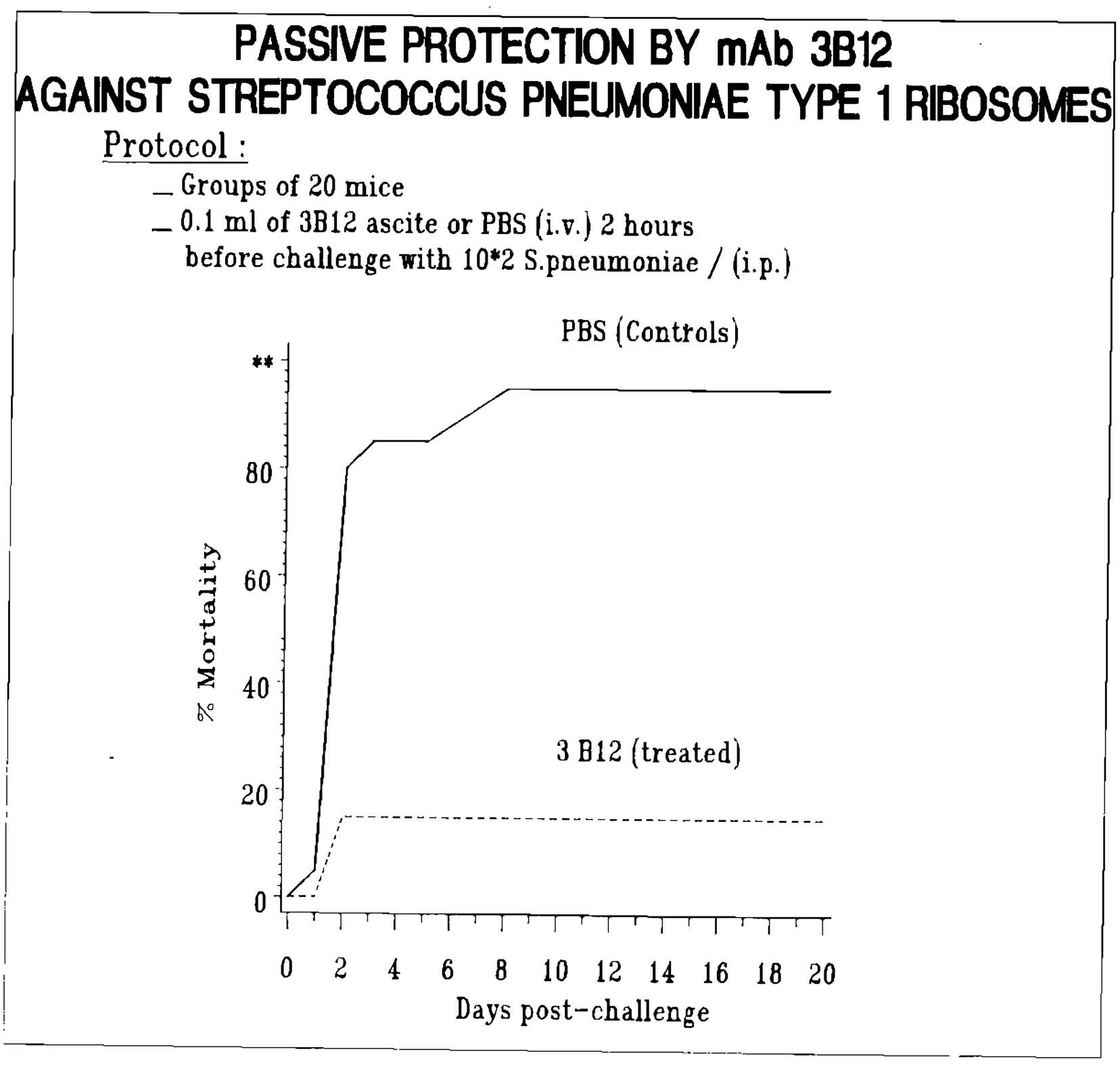

Fig. 4: Protective active of the MAb 3B12, raised against *Streptococcus pneumoniae* type I ribosomes, on mice challenged with *S. pneumoniae*.

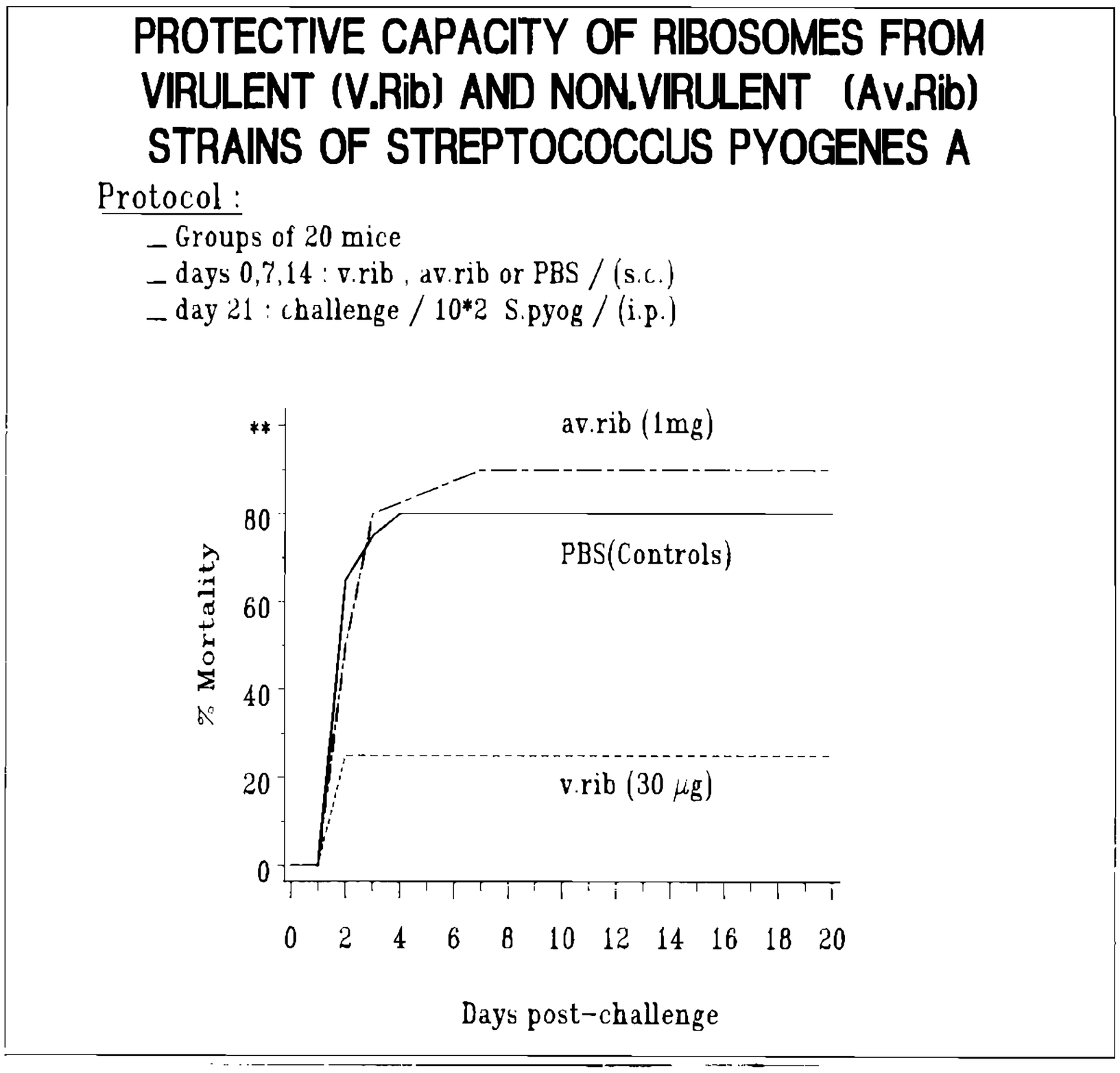

Fig. 5: Differences between the protective action of ribosomes from virulent or non-virulent *Streptococcus pyogenes* A strains.

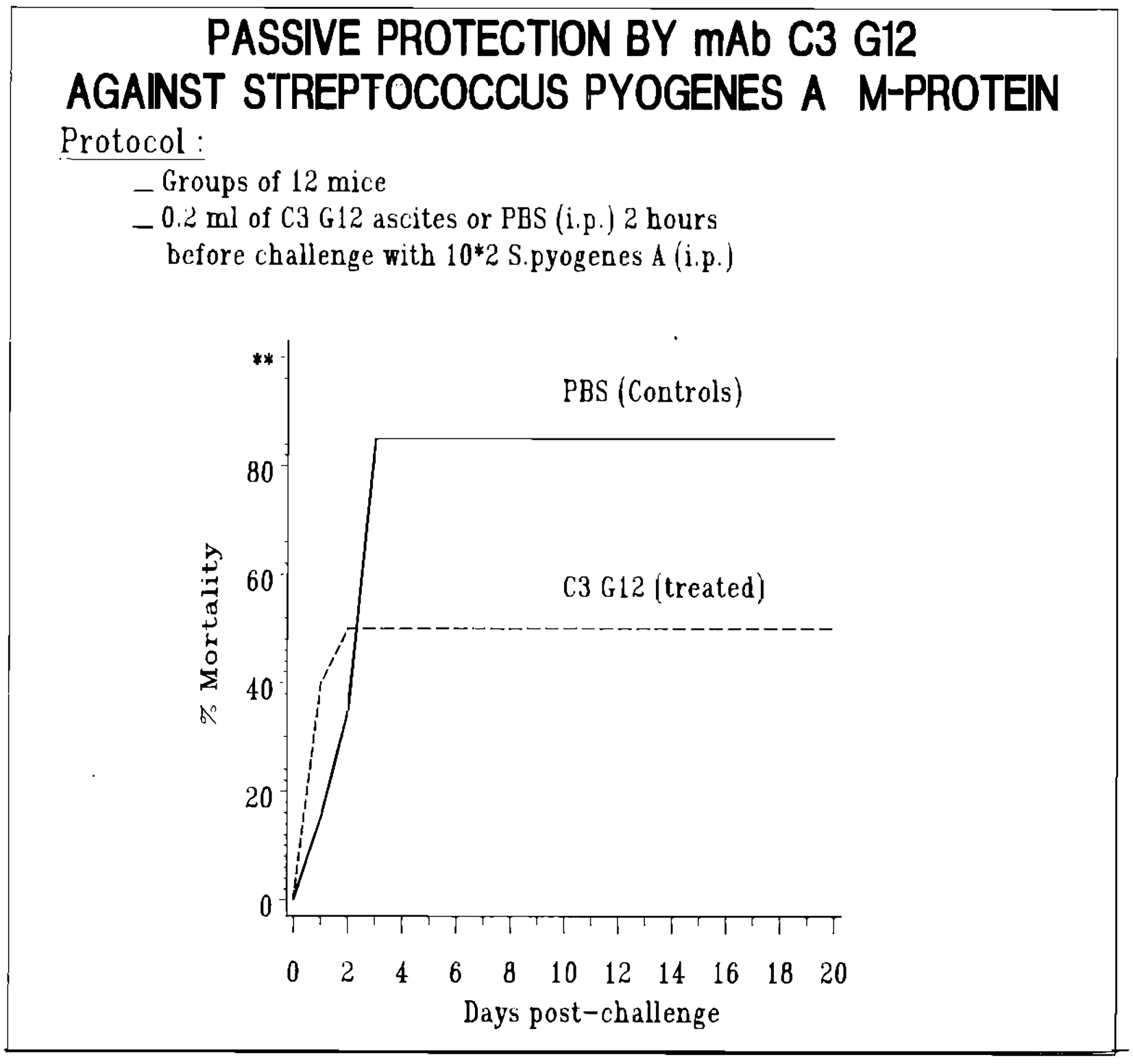

Fig. 6: Protective action of anti- *Streptococcus pyogenes A* M-protein MAb passively injected into Balb/c mice that were then challenged with *S. pyogenes A*.

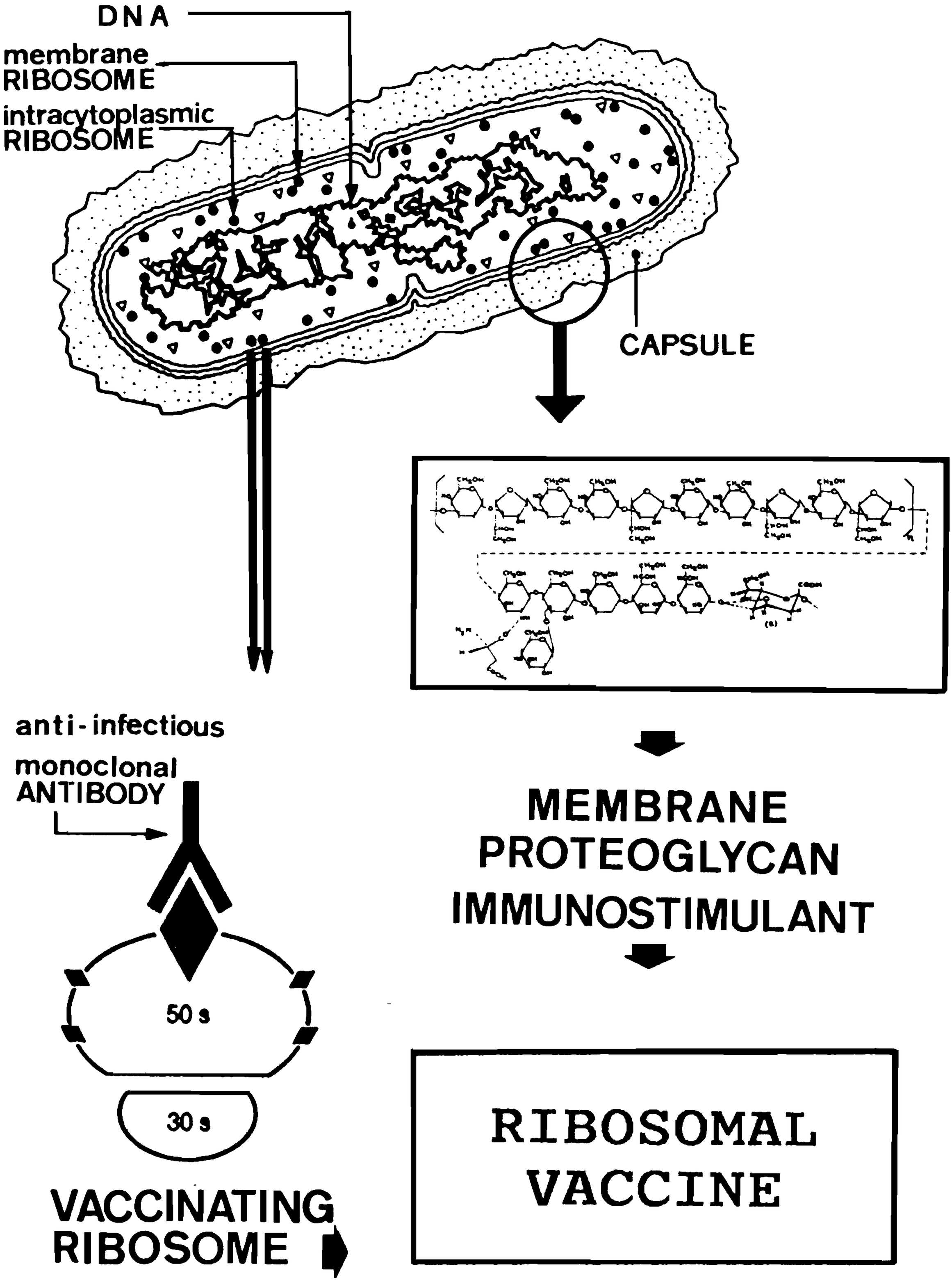

Fig. 7: General concept for obtention of ribosomal vaccines.

Membrane proteoglycan from:

– *Klebsiella pneumoniae* biotype a.15.0 parts

Not only bacterial ribosomes can be protective. We have also promising clinical studies under way with a preparation made of ribosomes isolated from *Candida albicans* type A and type B.

The formula of this preparation (D.651) is composed of:

Ribosomes from:

– *Candida albicans* type A . . . . . 3.5 parts
– *Candida albicans* type B . . . . . 3.5 parts

Membrane proteoglycan from:

– *Klebsiella pneumoniae* biotype a.15.0 parts

To finish I would just like to present briefly the protections obtained in mice with this vaccine or its separated components by the injectable and the oral route of administration.

Subcutaneous immunizations have been performed with 7 injections of 30 μg of M-PG kp, 6 μg of ribosomes or 22 g of the complete formula. Control group received PBS according to the same protocol.

Fig. 8 gives the protections obtained against a challenge with 1.4 x 10^5 *Candida albicans* type A (i.v.). One can see that the 60% of protection conferred by ribosomes alone are increased to 90% when associated to M-PG kp in the complete formula. M-PG kp was not protective by itself.

Fig. 9 shows similar results against a challenge with 1.0 x 10^5 *Candida albicans* type B (i.v.). In this case all the animals treated with D.651 survived the lethal infection.

Oral immunizations have been performed with 12 ingestions of 120 μg of M-PG kp, 28 μg ribosomes of 176 μg of the complete formula. Control group received PBS according to the same protocol.

Fig. 10 presents the protections obtained against a challenge with 1,4 x 10^5 Candida albicans type A (i.v.). Is this experiment 80% of mice treated with ribosomes or D.651 survived the challenge versus none in the control group.

Fig. 11 represents the protections conferred by oral route against a challenge with 1.0 x 10^5 *Candida albicans* type B (i.v.). One can see that 70% of mice treated with ribosomes and 100% of D.651 treated mice survived the challenge.

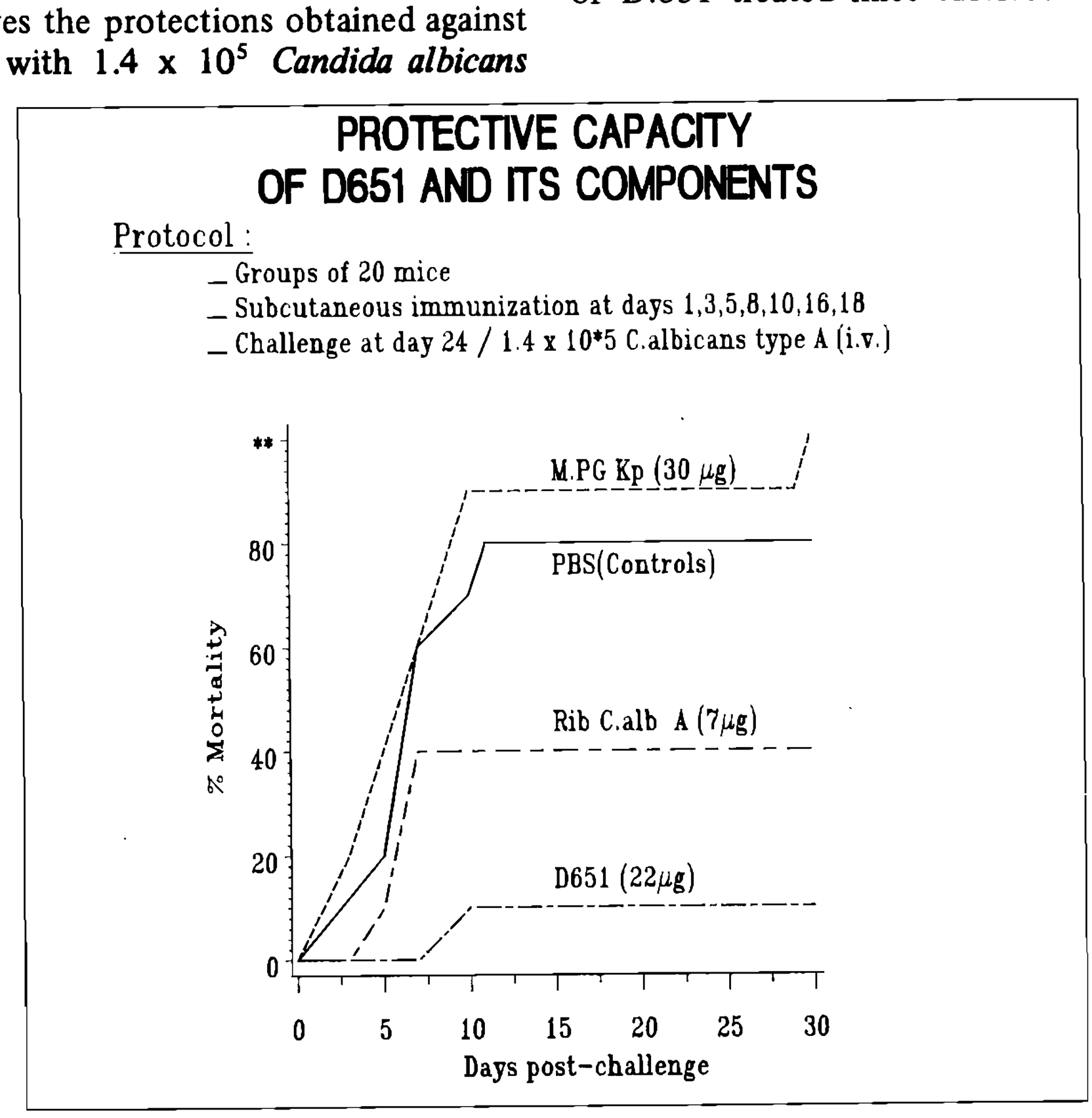

Fig. 8: Ability of the vaccine D 651 as well as its specific components to protect mice then challenged with *Candida albicans* type A or type B. This fig. depict the results obtained after subcutaneaus immunizations.

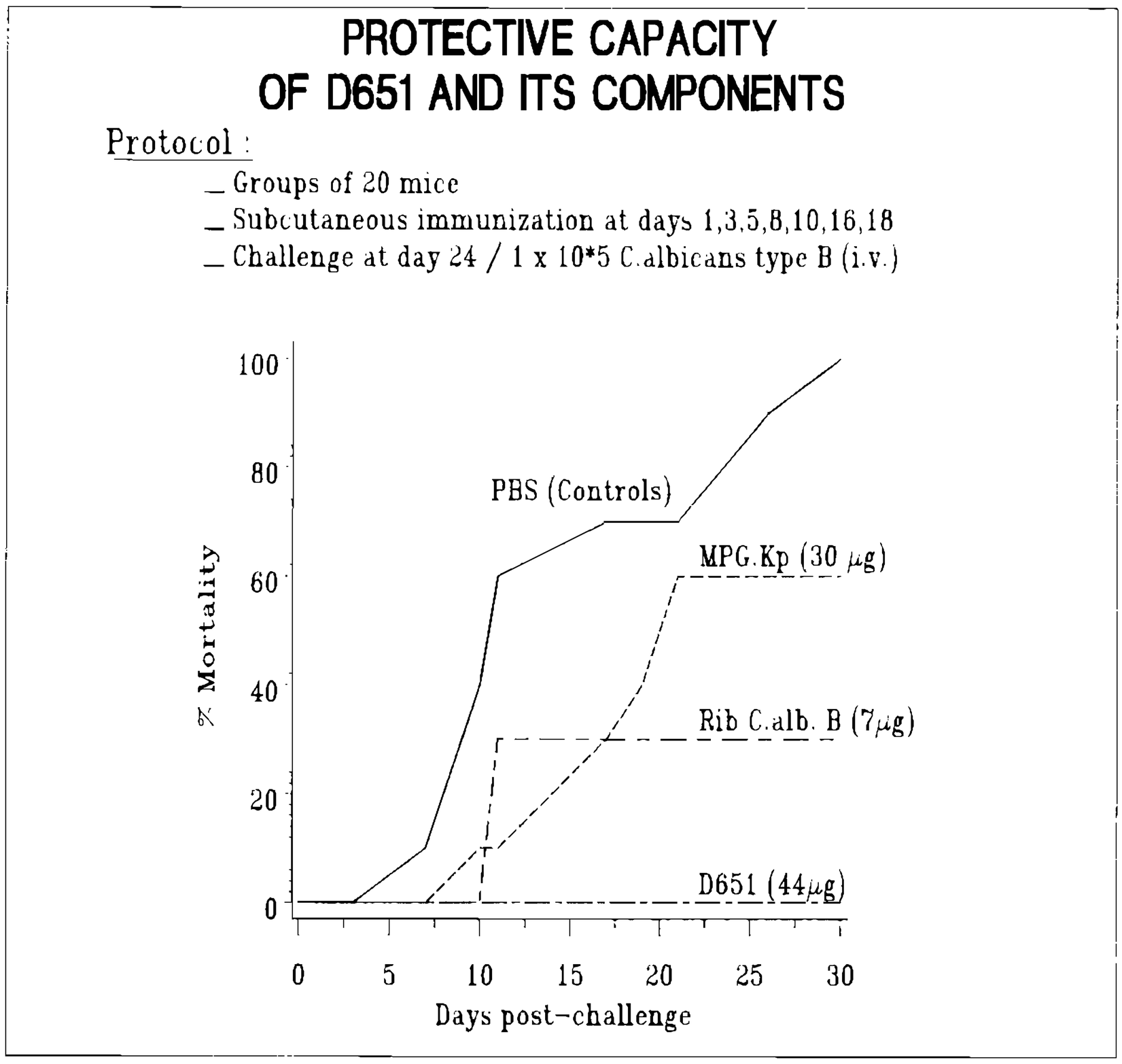

Fig. 9: Ability of the vaccine D 651 as well as its specific components to protect mice then challenged with *Candida albicans* type A or type B. This fig. depict the results obtained after subcutaneaus immunizations.

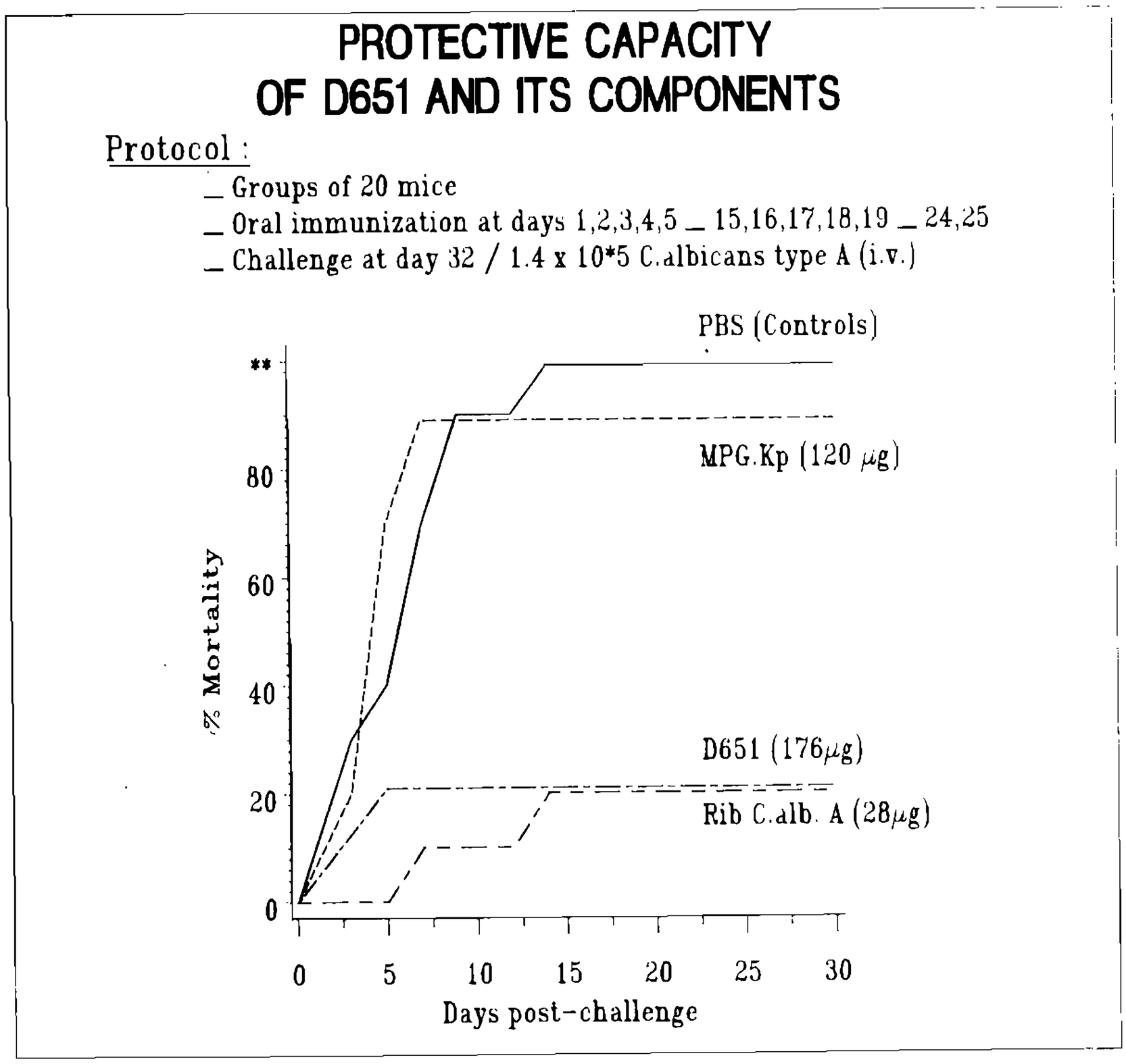

Fig. 10: Ability of the vaccine D 651 as well as its specific components to protect mice then challenged with *Candida albicans* type A or type B. The curves result from oral immunizations.

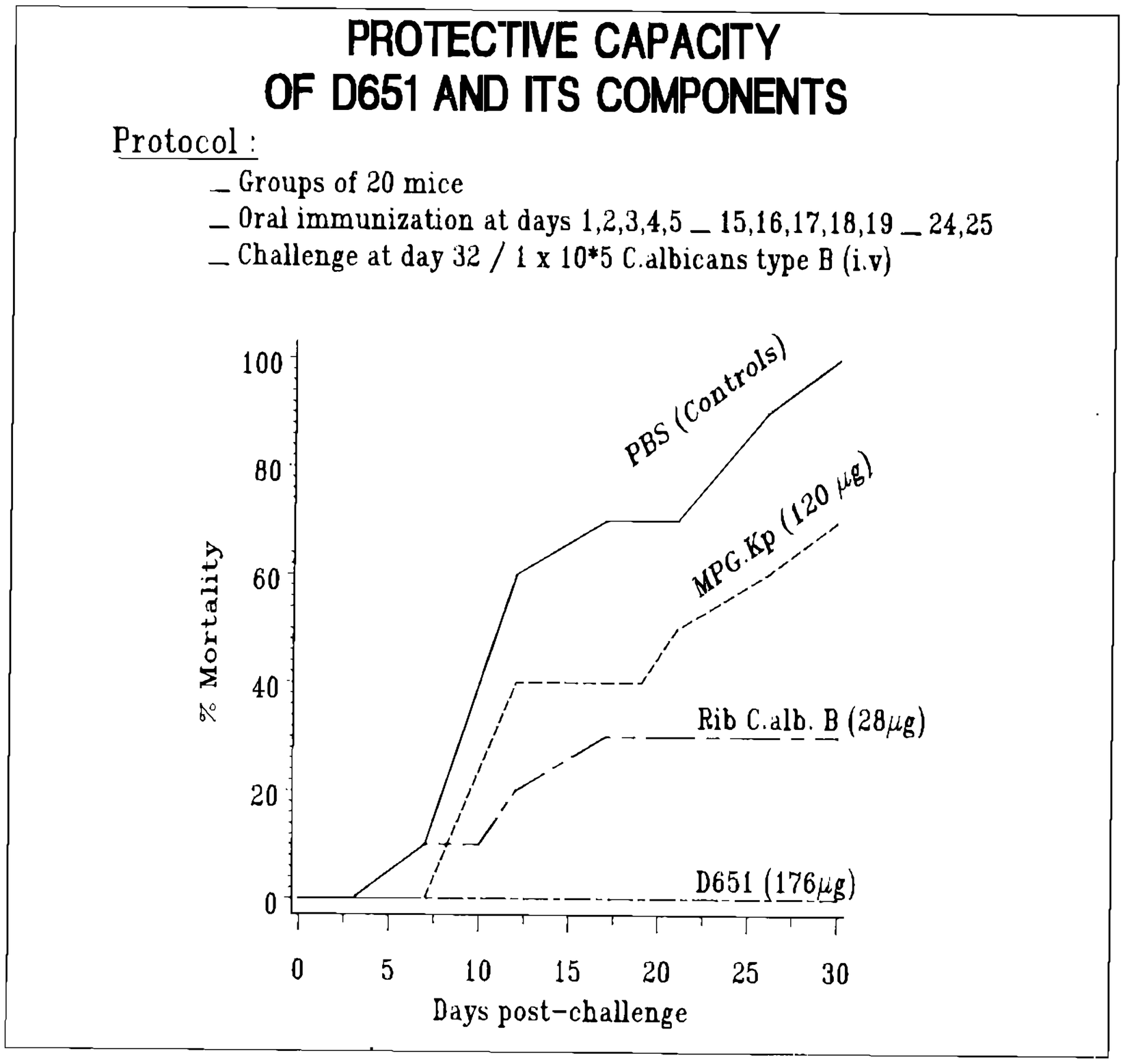

Fig. 11: Ability of the vaccine D 651 as well as its specific components to protect mice then challenged with *Candida albicans* type A or type B. The curves result from oral immunizations.

CONCLUSION

As a conclusion I would like to point out that this original concept of ribosomal immunotherapy represents a significant improvement over classical vaccines in many ways. They associates both specific and long term protection induced by ribosomes to the non-specific immunostimulant properties of the *Klebsiella pneumoniae* membrane proteoglycan (M-PG kp). They have a high chemical stability and can be produced industrially in very pure form for human use. Their efficiency and their innocuity is now well established.

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