recent findings that low molecular weight factors, probably proteins, are required for import of precursor proteins into mitochondria (*3–5*). Addition of unlabeled reticulocyte lysate to the pOTC-containing fractions substantially increased the import of OTC (Fig. 4B, lane 2). This stimulating activity of added lysate was abolished by prior treatment with trypsin (data not shown). Significantly, prior treatment of the added lysate with RNase did not abolish its import-stimulating activity (Fig. 4B, lane 3). Thus, while the 400-kD pOTC-containing fraction requires one or more additional protein factors for efficient import, it contains the RNase-sensitive component. Consistent with this interpretation is the finding that RNase treatment of both the precursor-containing 400-kD fraction and the added lysate was required to impair mitochondrial import of the fractionated material (Fig. 4B, lane 4). We conclude that the RNase-sensitive component either comigrates with or is part of the high molecular weight pOTC complex.

The results presented demonstrate that, in our cell-free reconstitution system, the posttranslational import of several mitochondrial matrix enzyme precursors by mitochondria is susceptible to inhibition by RNase. The mixing experiments (Fig. 3D) and the column fractionation experiments (Fig. 4) suggest that the RNase is not trivially generating a mitochondrial poison, but rather is acting on some as yet unidentified RNA component of the cytoplasm which copurifies with matrix enzyme precursors. Concentrations of RNase higher than those needed to degrade unprotected RNA's, and conditions that unfold protein-RNA complexes, are necessary to demonstrate inhibition, suggesting that the RNA involved must be tightly complexed with protein. We have been characterizing a subset of reticulocyte RNA's with the appropriate RNase susceptibilities, and are encouraged by the fact that 7*S*L RNA, known to be a necessary component of a cytoplasmic ribonucleoprotein (*7*), is one of these RNA species. Continuing studies with increasingly purified import systems should help elucidate the role of this putative ribonucleoprotein in the pathway of mitochondrial biogenesis.

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## Persistence of the Entire Epstein-Barr Virus Genome Integrated into Human Lymphocyte DNA

Abstract. *The entire Epstein-Barr virus genome is integrated into Burkitt tumor cell DNA at the terminal direct repeat sequence of the virus. There is no homology between the GC-rich (G, guanine; C, cytosine) terminal repeat and the AT-rich (A, adenine; T, thymine) cell sequences with which it has recombined. More than 15 kilobases of cell DNA have been deleted and 236 base pairs are duplicated at one virus-cell junction site.*

Most of the adult population is infected with Epstein-Barr virus (EBV) which is latent in a small fraction of B lymphocytes. The EBV causes infectious mononucleosis and is thought to be an etiologic agent of Burkitt lymphoma and nasopharyngeal carcinoma (*1*). The entire 170-kbp (kilobase pair) virus genome persists in latently infected cells (*2, 3*). Frequently, such cells have many EBV episomes (*4*), which are formed by covalent joining of the terminal direct repeats at both ends of the linear virion DNA (*5*). Analysis of cells for integrated EBV DNA is complicated by the presence of these episomes. Attempts to demonstrate covalent linkage of viral and cell DNA by isopycnic centrifugation (*6, 7*) and cell hybridization (*8*) have yielded conflicting data. In situ hybridizations have demonstrated that most or all of the EBV genome is linked to chromosomal sites 4q25 of the IB4 cell line and 1p35 of the Namalwa cell line (*9*). Namalwa is a latently infected African Burkitt tumor cell line and contains only one copy of EBV DNA (*9*). The IB4 cell line, established by infection and transformation of normal fetal lymphocytes with EBV, contains several episomal copies of EBV DNA in addition to the integrated copy (*9, 10*). We now describe the organization of the integrated EBV DNA in Namalwa and IB4 cells and the nucleotide sequence of the Namalwa integration sites.

To investigate the organization of EBV DNA in Namalwa cells, cloned Eco RI fragments representing more than 90 percent of the EBV genome (Fig. 1) were hybridized to Eco RI fragments of Namalwa DNA. Most probes hybridized to a single Namalwa cell Eco RI fragment of the appropriate size for an EBV DNA fragment. Thus, the organization of these fragments appears to be similar to that of linear viral DNA and these fragments are unlikely to have recombined with cell DNA. The EBV Eco RI D fragment consists of the joined

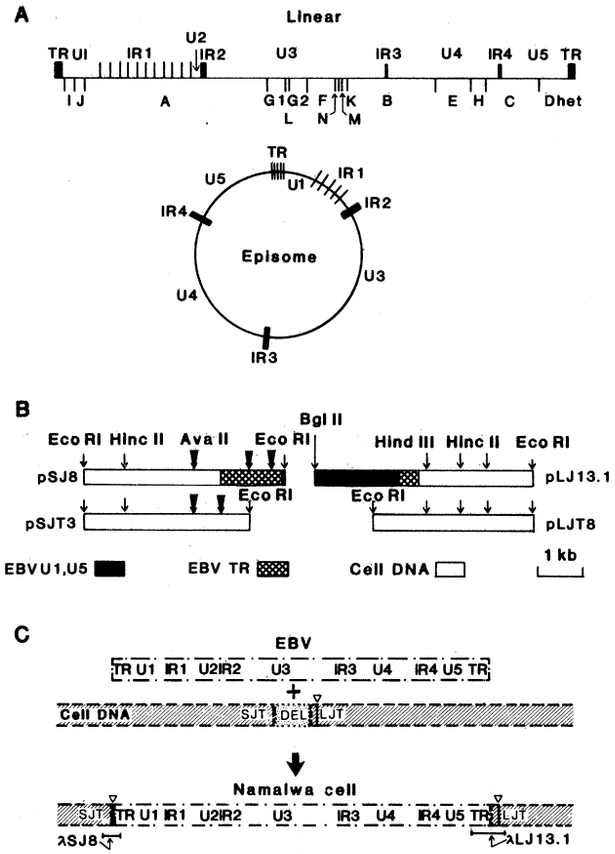


Fig. 1 (left). (A) EBV DNA (*5, 13, 18*). TR, terminal repeat region; U, single-copy sequence domain; IR, internal repeat; A to K, Eco RI fragments used as probes. (B) Recombinant clones. The Eco RI D probe includes the left and right terminal Eco RI fragments of linear virion DNA and hybridizes to two Namalwa Eco RI fragments designated LJ and SJ. The plasmid subclone pLJ 13.3 was derived from a lambda clone, LJ 13.1 of Namalwa LJ DNA. Similar designations are used for SJ and for clones of the homologous DNA's from the uninfected cells LJT and SJT. The procedures used in cloning DNA into lambda (*19*), in subcloning DNA into pUC and M13 (*20, 21*), and in sequencing (*22*), as well as the lambda clone library of normal cell DNA (*11*), have been described. Ava II restriction endonuclease sites are indicated by thick arrows; all other restriction endonuclease sites are indicated by thin arrows. (C) The Namalwa-EBV integration. "Del" indicates cell DNA deleted at the site of EBV integration. The dark vertical lines and triangles indicate the 236 base pairs of cell DNA which becomes duplicated at an EBV cell DNA juncture site. Fig. 2 (below). Sequence of the Namalwa-EBV junction sites (SJ and LJ) and of the unrecombined sites from normal cells (SJT and LJT). Cell DNA is shown in italic letters. The sequence shown begins in SJ and SJT cell DNA, proceeds through EBV TR to an Eco RI site in EBV U1, begins again in EBV U5, proceeds through EBV TR, and ends at the Hind III cell DNA site in LJ and LJT. The dark triangles show the limits of an Alu homologous region in SJ and SJT. Continuous underlines indicate the 236-base-pair part of LJT, which is duplicated at the junction between EBV TR and SJ cell DNA. In normal cell DNA and in Namalwa LJT DNA this sequence maps 0.8 kb 3′ to the Hind III site at the end of the LJ and LJT sequence shown. The dark arrows indicate junctions between EBV TR and cell DNA and open arrows indicate junctions between EBV TR and EBV U5 or U1. The single-dashed underlines indicate the 10-nucleotide direct repeat which brackets the EBV TR sequence and the double-dashed underlines indicate 22 nucleotides of TR which are homologous to a core sequence within the HSV "a" sequence (*17*). Dots indicate sites of variability among tandem copies of EBV TR in Namalwa. All sequences were analyzed for intra- and intersequence homology (*23*) and compared to the Genbank databank (*16*).

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SJT:      GTCCACCTCTACTGCCACTTTGCTTGACCTCAGCCTCTGTGAGTACTTCAGTGTCTCTGTAACATAGGGATGCTAATTACAGTCAGCTGCATTATTGCACATTCTGTGAACTGTGCAGTG
SJ:     1 GTCCACCTCTACTGCCACTTTGCTTGACCTCAGCCTCTGTGAGTACTTCAGTGTCTCTGTAACATAGGGATGCTAATTACAGTCAGCTGCATTATTGCACATTCTGTGAACTGTGCAGTG

SJT:      CATAGGGAACATATCACCATTATGGCATTAGAGAATGCCCTTCCTAAAACATTTAAAATGTCACTTCCTTCAACTGCAATGCAATTTCAAAGTTTTCCTCTTATATCTTTTTTTTTTTTT
SJ:   121 CATAGGGAACATATCACCATTATGGCATTAGAGAATGCC.TTCCTAAAACATTTAAAATGTCACTTCCTTCAACTGCAATGCAATTTCAAAGTTTTCCTCTTATATCTTTTTTTTTTTTT

SJT:      TCCTGGCAGAGTTTGCTCAGAAGTGAATTTTTTTTTTTTTTTTTTTTTGAGATGGGGTCTCACTCTGTCACCCAGGCTGGAGTGCAGTGGCACGATCTCGGCTCACTGCAGCCTCCGCC
SJ:   241 TCCTGGCAGAGTTTGCTCAGAAGTGAATTTTTTTTTTTTTTTTTTTT.GAGATGGGG.CTCACTCTGTCACCCAGGCTGGAGTGCAGTGGCACGATCTCGGCTCACTGCAGCCTCCGCC

SJT:      TCCTGGGTTCAAGTGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGGACTACAGGCATGCACCAACATGCCCAG.....................................................
LJT:                                GACCGCGCCCACCATACAGATTACAGAGGTAAGGTACCCATCCCCATTGG.ACTAGGGTGTTCTTATAATGCTGAAAATTGAGGTCATTACCT
SJ:   361 TCCTGGGTTCAAGTGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGGACTACAGGCATGCACCAACATCCCCATTGGGACTAGGGTGTTCTTATAATGCTGAAAATTGAGGTCATTACCT

SJT:      .....................................................................................................................
LJT:      GTAAGGGAATTTCCCTTAACTTTAGTGGAATAAATTTAATTAGTGCAGTTTATTATGCCAGTTTCATTTGGGGTGGAACCTAGAAGTGAGAGAGTGATGGGAAGTGATGTTGTTAAGGAA
SJ:   481 GTAAGGGAATTTCCCTTAACTTTAGTGGAATAAATTTAATTAGTGCAGTTTATTATGCCAGTTTCATTTGGGGTGGAACCTAGAAGTGAGAGAGTGATGGGAAGTGATGTTGTTAAGGAA

SJT:      .....................................................CTAAATTTTTTATTTTAGTAGAGACGGGGTTTTGCCATGTTGGCCAAGCTGGTCTTGAACTCCTGGC
LJT:      TCCCACCATGTTTTTTCTATAGGACACTTGAAATTGATAAGCCTTTCTTTAACTAAAACCAGAGTTGGTTTGAGATAGATTTTTTCTAGGAGGCATGCTTATCTGTTTGCTCTTTCTCTC
SJ:   601 TCCCACCATGTTTTTTCTATAGGACACTTGAAATTGATAAGCCTTTCTTTAACTAAAACAGTGCGGGCGGTGGATGGCGGCTGGGGTTCAGGGGAACGGGGGGGTCGGGGGGCGCTGCGG
NTR:      GCACAGAGCG.CGCAACGGCGGGCGGGCGGCGGGGGGTCGGGGTCCGCGG.CTCCGGGGGCTGCGGGCGGTGGATGGCGGCTGGGGTTCAGGGGAACGGGGGGGTCGGGGGGCGCTGCGG

SJT:      CTCAGTTGATCTACCTGCCTTGGCCTCCCAAAGTGCTGGGATTACAGACATGAGCCACTTCGCCCGGCCAAGGTCATGTATTGTTAGGCTTAGGAGGAAGAAACACTGGGGG
SJ:   721 CCGCGCAGCCATGCGTGACCGGAGGGGGGACAGGGTCGCAGGGGGCGTGTCTGGTGGGGGCGGGAGCGGGGGGCGGCGCGGGGAGCCTGCACGCCGTTGGAGGGGAGAATGACAGGGGGG

SJ:   841 CGGGGACAGAGAGGCGGTCGCGCCCCGGCCGCGGCAGCCAAGCCCCCAAGGGGGGCGGGGCGGGCAGCGGAGGCACGGTGACGAAGGGCCCCAGGGCTGACCCCGGCAAACGTGACCCGG

SJ:   961 GCCTCCGGGGTGACCCAGCCAAGCGTGACCAAGGGGCCGCTGGGTGACACAGACAACCCTGACAAAGGCCCCCCAGGAAAGACCCCCGGGGGGGCTTCGGGGGGTGTTGGAGAGGGCATGG

SJ:  1081 GGGG(AGCATGGGCCGCCGCGCATTCCTGGAAAAAGTGGAGGGGGCGTGGCTGGCCCCCGCGGCCCCTAGCCCCCCCGCACAGAGCGCGCAACGGCGGGCGGGCGGCGGGGGGTCGGGGT

SJ:  1201 CCGCGGGCTCCGGGGGCTGCGGGCGGTGGATGGCGGCTGGGGTTCAGGGGAACGGGGGGGTCGGGGGGCGCTGCGGCCGCGCACGCATGCGTGACCGCGAGGGGGGACAGGGTCGCAGGG

SJ:  1321 GGCGTTGTCTGGTGGGGCGGGAGGGGGGCGCGGGGAGCCTGCACGCCGTTGGAGGGGAGAATGACAGGGGGGCGGGGACAGAGAGGCGGTCGCGCCCCGGCCGGCAGCAAGCCCCCAAGG

SJ:  1441 CGGCGCGGGCACGGACGTGACGAAGGGCCCCAGGGCTGACCCCGGCAAACGTGACCCGGGCCTCCGGTGACCCAGCCAAGCGTGACCAAGGGGCCGCTGGGTGACACAGACAACCCTGAC

SJ:  1561 AAAGGCCCCCAGGAAAGACCCCCGGGGGGGCTTCGGGGGGTGTTGGAGAGGGCATGGGGGG)3GCGGATTT.CGCCCTTATTGCCCTGTTTAGAATTC U1-IR1-U2-IR2-U3-IR3-U4-IR4-U5

LJ:     1 CAGCAGACGGCGGATATGGGAATTTCAGAATGAGGTGGCGGATTCAGGCGAAAAGGGTGTGGGCTGTGCGAGAGTCATGAGGCAGGCGCGGAAAGTCGCTGCGGCTTGCAGCATGGGGGG

LJ:   121 (TR)9 AGCATGGGCCGCCGCGCATTCCTGGAAAAAGTGGAGGGGGCGTGGCCTGGCCCCCCGGCGGCCCCCTAGCCCCCCCGCACAGAGCGGCGCAACGGCGGGCGGGCGGCG

LJ:   241 GGGGGTCGGGGTCCGCGGGCTCCGGGGGCTGCGGGCGGTGGATGGCGGCTGGGGTTCAGGGGAACGGGGGGGTCGGGGGGCGCTGCGGCCGCGCACGCCATGCGTGAACCGCGAGGGGGG

LJT:      GTGAGATGCTGGGCCTTGCTGGTTTTTAACAATTTTGTTTGTTCCTCTTAAAGTTGATATTAGCTCTGATCAAAGTATCTAAATACATAAACTGGTATAAAAGTACTCATTATGGTTTAA
LJ:   361 ACAGGGTCGCAGGGGGCGTGTCTGGTGGGGGCGGGA.CGGGGGGCGGC.CGGGGAGCCTGCACGCCGTTGGAGGGGAGAATGACAGGGGGGCGGGTATAAAAGTACTCATTATGGTTTAA
NTR:      ACAGGGTCGCAGGGGGCG.GTCTGGTGGGGGCGGGA.CGGGGGGCGGC.CGGGGAGCCTGCACGCCGTTGGAGGGGAGAATGACAGGGGGGCGGGGACAGAGAGGCGGTCGCGCCCCGGC

LJT:      TTCTTTTTTTACCTAGCAATTAAGGGAATCTATTGCTGAACACAAACCTCATATTGACAAACTACTAAAGATAGGCCCACAACTAAAGGAATTAAACCCTGAGGAAGGGGAAATGGTGGA
LJ:   481 TTCTTTTTTTACCTAGCAATTAAGGGAATCTATTGCTGAACACAAACCTCATATTGACAAACTACTAAAGATAGGCCCACAACTAAAGGAATTAAACCCTGAGGAAGGG.AAATGGTGGA

LJT:      AGAAAAATACCAGAAAGCAGAAAACATGTATGCCCAAATAAAGGAGGAGGTGCGCCAGCGAGCCCTGGCTCTGGATGAAGCCGTGTCCCAGTCCACACAGGTATGTGTGTCTCGACACT
LJ:   601 AGAAAAATACCAGAAAGCAGAAAACATGTATGCCCAAATAAAGGAGGAGGTGCGCCAGCGAGCCCTGGCTCTGGATGAAGCCGTGTCCCAGTCCACACAGGTATGTGTGTCTCGACACT

LJT:      CATAACCTTGAATTGTCTACTGTTCTAAACAAGAGGTCTCTGTATCAGGTATGTCTCTGTGCTCAGGAATGTCCAGAGCAGCATGTTAATACGTGATCGGTAGATAGAACAGAAAGCT
LJ:   721 CATAACCTTGAATTGTCTACTGTTCTAAACAAGAGGTCTCTGTATCAGGTATGTCTCTGTGCTCAGGAATGTCCAGAGCAGCATGTTAATACGTGATCGGTAGATAGAACAGAAAGCT
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right and left terminal Eco RI fragments of cloned episomal EBV DNA (*5*) (Fig. 1). The terminal repeat (TR) sequence which is at the ends of linear virion EBV DNA is contained within the episomal Eco RI D fragment (Fig. 1). The Eco RI D probe hybridized to two Namalwa Eco RI fragments, 5 and 14 kb in size (designated SJ and LJ, respectively). Two terminal fragments were also detected when hybridized with Bam HI, Bgl II, or Pst I fragments of Namalwa DNA. Both terminal fragments hybridized to EBV TR. One fragment hybridized to a probe from the single-copy DNA at the right end of the EBV genome (U5), while the other hybridized to single-copy DNA from the left end of the EBV genome (U1). No Namalwa fragment hybridized to both U1 and U5 probes. Thus, in Namalwa cells the ends of EBV DNA are not joined to each other and may have recombined with cell DNA.

Parallel experiments demonstrated that most IB4 EBV Eco RI fragments are also comparable in size to linear viral DNA. Two types of fragments are evident in IB4 DNA, which are not found in Namalwa DNA. One type originates from EBV episomes in IB4 cells. The second type appears to be an EBV episome that has integrated into cell DNA at a point within Eco RI fragment I. This conclusion is based, for example, on observations that the Eco RI I probe hybridized with three IB4 Eco RI fragments that were larger, smaller, and the same size as EBV Eco RI I. In all of the IB4 EBV DNA molecules, the single-copy DNA's at the right and left ends of the EBV genome were linked through TR.

Next, two independent lambda clones of putative Namalwa junction fragment LJ and four independent clones of SJ were made and subcloned into pUC plasmids. The subcloned DNA's were then used to select at least three independent uninfected cell DNA clones (designated LJT and SJT, respectively) from a library of human cell DNA clones constructed in lambda vectors (*11*). The LJT and SJT clones were again subcloned into pUC plasmids. Restriction endonuclease mapping of the EBV DNA in Namalwa LJ and SJ clones indicated that part of each of these DNA's was identical to the right and left ends of linear EBV DNA, respectively (Fig. 1B). The rest of the LJ and SJ cloned DNA's was identical to normal cell DNA in the LJT and SJT clones (Fig. 1B). Each LJT and SJT clone contained 15 to 20 kb of uninfected cell DNA which included part of the cell DNA of the Namalwa junction with which it was selected. However, there is no overlap between LJT and SJT cell DNA's. Therefore, cell DNA sites with which the right and left ends of the EBV genome have recombined (represented as LJT and SJT, respectively) are more than 15 kb apart in uninfected cell DNA. At least 15 kb of uninfected cell DNA is deleted at the Namalwa EBV integration site. The results are summarized in Fig. 1C.

The nucleotide sequence of the junction sites between EBV and Namalwa DNA (SJ and LJ) and of the uninfected cell DNA sites (SJT and LJT) were compared (Fig. 2). The sequence of cell DNA from the Namalwa SJ clones matched the sequence from uninfected cell SJT clones almost perfectly, as was true for the match between the sequences of LJ and LJT. There were 3.8 copies of EBV TR between the left end of single-copy EBV DNA and cell DNA and 9.6 copies of EBV TR between the right end of EBV DNA and cell DNA. There was no homology between EBV TR and the cell DNA's with which it had recombined. The region of cell DNA at which recombination took place was unusually AT-rich; 75 percent of the 50 nucleotides of cell DNA adjacent to the junction were A or T. The sequence TAAA occurred in cell DNA at both sites of recombination. A 236-bp cell DNA sequence which mapped at a distance of 1.2 kb from the LJ site was duplicated at the SJ site. The same sequence was found in uninfected LJT DNA but not in SJT DNA. Oligonucleotide homology (CTAAA) was noted between SJ cell DNA and the boundaries of the duplicated sequence.

The only previous example of stable herpesvirus DNA integration into cell DNA is a small segment of equine herpesvirus DNA found in virus-transformed cells (*12*). In EBV infection, there could be selection for those cells which maintain the entire EBV genome, as widely separated regions of the genome encode viral proteins presumably necessary for virus-induced cell proliferation and maintenance of the virus in a latent state (*13*). Integration is the sole mechanism of intracellular virus persistence in Namalwa cells. In contrast, IB4 cells contain integrated and episomal EBV DNA. An unusual property of Namalwa cells which might be correlated with the absence of EBV episomes is the lack of virus replication in response to inducers (*14*).

Integration could be directly relevant to the oncogenic properties of EBV if it has an effect on viral or cellular gene expression, as has been demonstrated with retroviruses. *Blym* and c-*myc* are two cellular genes that are activated in Burkitt tumor cells. *Blym* maps near the site of EBV integration in Namalwa (*15*). We can find no homology between cloned *Blym* and cloned cell DNA from around the sites of EBV integration in Namalwa cells. Moreover, comparison of the cell DNA sequence (*16*) with those stored in the Genbank databank as of March 1984 revealed no significant homology other than the Alu sequence indicated in Fig. 2.

In the Namalwa system, integration of EBV could be site specific for a sequence within TR. In considering a possible specific cleavage within the EBV TR sequence, we note that it is similar to the herpes simplex virus terminal repeat "a" sequence, which is known to be specifically cleaved for isomerization and packaging (*17*). Both sequences are GC-rich, have oligodeoxyguanylate and deoxycytidylate sequences, and are bracketed by a short terminal direct repeat at their juncture with a single-copy viral DNA sequence (Fig. 2). The short direct repeat is also repeated within the TR and "a" sequences. Also within TR, near the EBV short terminal direct repeat, is the sequence GGCCCCCAGGAAAGACCCCCGG, which is homologous to a highly conserved domain of the herpes simplex "a" sequence (*17*).

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## Monoclonal Idiotope Vaccine Against *Streptococcus pneumoniae* Infection

Abstract. *A monoclonal anti-idiotope antibody coupled to a carrier protein was used to immunize BALB/c mice against a lethal* Streptococcus pneumoniae *infection. Vaccinated mice developed a high titer of antibody to phosphorylcholine, which is known to protect against infection with* Streptococcus pneumoniae. *Measurement of the median lethal dose of the bacteria indicated that anti-idiotope immunization significantly increased the resistance of BALB/c mice to the bacterial challenge. Antibody to an idiotope can thus be used as an antigen substitute for the induction of protective immunity.*

The network hypothesis of Jerne (*1*) defines the immune system as a web of interacting idiotopes. Idiotopic markers are found on the variable regions of both immunoglobulins and T-cell receptors (*2–4*). Inherent in the network theory is the postulate that external or nominal antigens are mimicked by idiotope structures. If these internal antigens could be exploited as antigenic structures, an alternative set of antigens would be available for vaccine preparations that could complement conventional vaccines.

Idiotope vaccines offer distinct advantages over conventional vaccines derived from nominal antigens, which often require elaborate and expensive purification. Idiotope vaccines can be easily prepared by producing hybridoma anti-idiotope antibodies and coupling them to immunogenic carrier molecules. Biological advantages arise from the fact that idiotope antigens exist in a different molecular environment from that of nominal antigens, which allows an idiotope vaccine to stimulate silent or tolerized immune clones (*5, 6*). The latter point bears on the issue of tumor antigens to which an acquired state of tolerance often exists. If T cells are "educated" by idiotypes and internal images (*7, 8*), the antibody to the idiotope may elicit effective T-cell help through associative recognition.

We now report the use of a modified monoclonal anti-idiotope antibody as a vaccine against a virulent bacterial infection in BALB/c mice. Our approach is based on data showing that the TEPC 15 (T15) idiotype, an antibody specific for phosphorylcholine (PC), is effective in protecting mice against a lethal *Streptococcus pneumoniae* infection (*9, 10*). We selected the binding site–specific hybridoma antibody 4C11 from a set of monoclonal A/He anti-T15 antibodies prepared previously (*11*). The 4C11 antibody was coupled to keyhole limpet hemocyanin (KLH) with glutaraldehyde (*12*).

Normal BALB/c mice were used in these experiments because this strain produces a dominant T15 idiotype (*13*). The mice were injected twice during a 1-month period (at 2-week intervals) with doses (100 μg each) of KLH, PC-KLH, 4C11, 4C11-KLH, 315, or 315-KLH (315 is a nonrelated idiotype). One week after the second injection, the mice were bled and their sera tested for antibodies to PC and for the T15 idiotype by an ELISA (enzyme-linked immunosorbent assay) and by radioimmunoassay. The results (Table 1) showed that only PC-KLH and 4C11-KLH caused significant elevations of antibody to PC. Used alone as an immunogen, 4C11 also increased the level of antibody to PC though not to that achieved by 4C11-KLH or PC-KLH.

Type 3 virulent *S. pneumoniae* (American Type Culture Collection) was grown on Trypticase soy agar supplemented with 10 percent sterile sheep red blood cells. Normal and immunized BALB/c mice were challenged with $10^4$, $10^5$, $10^6$, $10^7$, or $10^8$ *S. pneumoniae* administered intravenously; these doses were based on the results of preliminary $LD_{50}$ (median lethal dose) experiments. Deaths were recorded on a daily basis for 7 days (Table 2). The $LD_{50}$ dose for normal, unprimed BALB/c mice is approximately $4 \times 10^5$ bacteria, a relatively large number (*10*) resulting from the naturally high concentration of T15 idiotype in the

Table 1. Serum response of BALB/c mice to nominal and internal antigens. The mice received intraperitoneal injections (100 μg each) of the immunogens listed suspended in complete Freund adjuvant; 2 weeks later they received a booster injection of the same immunogen in incomplete Freund adjuvant. Antibodies to PC were determined by an ELISA; concentrations (± standard error of the mean) were measured by comparison to a standard. The amount of T15 idiotype (>92 percent in all cases) in the antibody to PC was determined by means of $^{125}$I-labeled monoclonal anti-T15 antibody in a radioimmunoassay; $n$ = about 20 mice per group.

| Immunogen | α-PC in serum (μg/ml) |
|---|---|
| Control | 35.6 ± 6 |
| 315 | 25 ± 2 |
| 315-KLH | 70 ± 15 |
| KLH | 81 ± 27 |
| PC-KLH | 8800 ± 2500 |
| 4C11 | 542 ± 184 |
| 4C11-KLH | 5072 ± 675 |

Table 2. Protection of BALB/c mice against *Streptococcus pneumoniae* infection by nominal and idiotope vaccines. Mice were immunized as described (see Table 1) and challenged with serial dilutions (tenfold) of *S. pneumoniae* administered intravenously 5 days after the second injection of immunogen. Deaths were recorded daily for 7 days. The $LD_{50}$ was estimated as described (*19*). Log (protection) indicates the relative amount of protection for immunized animals compared to normal unprimed controls [log (protection) = 0]. The number of survivors is recorded for the $10^6$ *S. pneumoniae* challenge dose; S.E.M., standard error of the mean.

| Immunogen | $LD_{50}$ of *S. pneumoniae* (± S.E.M.)* | Log (protection) | Survivors (No.) |
|---|---|---|---|
| Control | $3.8 \times 10^5$ ($1.7 \times 10^5$) | 0 | 9 of 28 |
| KLH | $1.9 \times 10^5$ ($1.6 \times 10^5$) | −0.3 | 3 of 10 |
| PC-KLH | $1.9 \times 10^7$ ($1.4 \times 10^7$) | +1.7 | 7 of 9 |
| 315-KLH | $1.1 \times 10^6$ ($1.4 \times 10^6$) | +0.4 | 6 of 11 |
| 4C11 | $1.4 \times 10^6$ ($5 \times 10^5$) | +0.6 | 10 of 19 |
| 4C11-KLH | $2.3 \times 10^7$ ($6.6 \times 10^6$) | +1.8† | 30 of 32 |

*Standard errors were calculated on the basis of $n = 3$. †$P < 0.01$ compared to control ($t$-test).