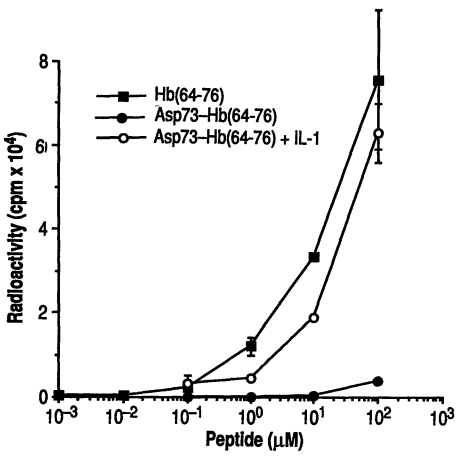

Fig. 3. The Asp73–Hb(64-76) peptide and exogenous IL-1 costimulate the 2.102 Th2 cells to proliferate. The 2.102 Th2 cells (10^5) were cultured with APCs, peptide, and tritiated thymidine as described in Fig. 1. Murine rIL-1β (100 pg/ml) was added to additional wells containing the Asp73–Hb(64-76) peptide and APCs. One of three experiments is shown. In this experiment, 3900 cpm were recorded from the wells containing rIL-1, APCs, and Th2. Addition of rIL-1 to cultures containing the 2.102 Th2 cells and monoclonal antibody to CD3 (145-2C11) in the absence of APCs caused the T cells to proliferate (53,300 cpm). Results are reported as the mean ± SD of triplicate cultures.

quired signal, in addition to recognition of antigen by the TCR, that leads to T cell proliferation (*19, 20*). The altered ligand apparently modifies the interaction between T cell and APC such that proper activation of the APC (*21*) or the reception of signals by the Th cell does not occur (*20*). If costimulation depends on the strength of interaction between TCR and ligand, then activation of T cell cytokine production may be more permissive than expansion of Th cells by proliferation. When an immune response to antigen is evaluated, it may be necessary to consider functional aspects of T cell activation, such as help, because proliferation may not properly reflect the entire response. Differences between the antibody and T cell proliferative responses have been seen with other antigens (*6, 22*). In one case (*6*), changing the MHC molecule and possibly the TCR ligand led to separate responses. Whereas those studies used bulk populations of T cells, in our study it was possible to dissociate the responses of the TCR of a cloned population of Th cells.

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23. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Induction of *Plasmodium falciparum* Transmission-Blocking Antibodies by Recombinant Vaccinia Virus

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Many candidate antigens of malaria vaccines have limited immunological recognition. One exception is Pfs25, a cysteine-rich, 25-kilodalton sexual stage surface protein of *Plasmodium falciparum*. Pfs25 is a target of monoclonal antibodies that block transmission of malaria from vertebrate host to mosquito vector. The surface of mammalian cells infected with a recombinant vaccinia virus that expressed Pfs25 specifically bound transmission-blocking monoclonal antibodies. Furthermore, major histocompatibility complex–disparate congenic mouse strains immunized with recombinant Pfs25 elicited transmission-blocking antibodies, demonstrating that the capacity to develop transmission-blocking antibodies is not genetically restricted in mice. Live recombinant viruses may provide an inexpensive, easily administered alternative to subunit vaccines prepared from purified recombinant proteins to block transmission of malaria in developing countries.

THE SPREAD OF MALARIA CONTINues, despite intensive chemotherapeutic intervention and vector control campaigns. One of several alternatives currently under investigation to slow or reverse the increasing epidemic of malaria is a transmission-blocking vaccine (*1*). Transmission of *P. falciparum* from host to mosquito vector can be blocked by monoclonal antibodies (MAbs) to a 25-kD sexual stage surface protein, Pfs25, expressed on zygotes and ookinetes (*2*). The gene encoding Pfs25 has been cloned (*3*). The deduced amino acid sequence revealed a striking feature: the presence of four tandem epidermal growth factor (EGF)–like domains. EGF-like domains are cysteine-rich and depend on proper disulfide bond formation for structural integrity (*4*). It is not surprising, therefore, that, of the MAbs known to block transmission, none recognize the reduced Pfs25 antigen (*2, 5*), suggesting that, for at least some of the blocking epitopes, disulfide bonds are involved in creating proper conformation.

A subunit vaccine for controlling endemic malaria in developing countries must induce high, long-lasting antibody titers and must be produced in large amounts at the lowest possible cost. Bacteria or yeast provide a simple means of recombinant protein expression that is inexpensive, if the recombinant products can be easily purified and are immunologically effective. Live attenuated viruses, such as vaccinia or adenovirus, are an attractive alternative because they are inexpensive to produce and are easily transported and administered.

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Furthermore, because the antigen is produced in the mammalian host's cells, proper folding and posttranslational modifications are more likely to occur in this expression system than in prokaryotic expression systems. In this study, we used recombinant vaccinia virus, containing the gene coding for Pfs25, as a live, attenuated virus vector to express Pfs25 in mammalian cells in vitro and to inoculate mice for immunogenicity and efficacy studies.

We inserted the full-length gene encoding Pfs25 into the vaccinia virus genome (strain WR) by homologous recombination with a transfer vector, pTKgpt-OF1S. The recombinant virus, vSIDK, was isolated and used for the infection of mammalian BSC-1 cells (*6*). Recombinant Pfs25 (rPfs25) expression resulted in proper folding of epitopes for MAbs 1C7 (Fig. 1, C and D), 1D2, and 32F61 (*2, 3*), all of which recognize reduction-sensitive epitopes. Furthermore, in striking contrast to *Plasmodium* circumsporozoite protein (*7*), rPfs25 appeared on the surface of infected mammalian cells. In indirect immunofluorescence, MAb 1C7 gave a uniform, strong surface fluorescence reaction with vSIDK-infected BSC-1 cells (Fig. 1, C and D); however, WR-infected cells were completely negative in reaction with MAb 1C7 (Fig. 1, A and B), and so were vSIDK-infected cells examined with normal mouse sera (*8*). The reaction with MAb 1C7 was stronger 48 hours after infection than at 24 hours (*8*).

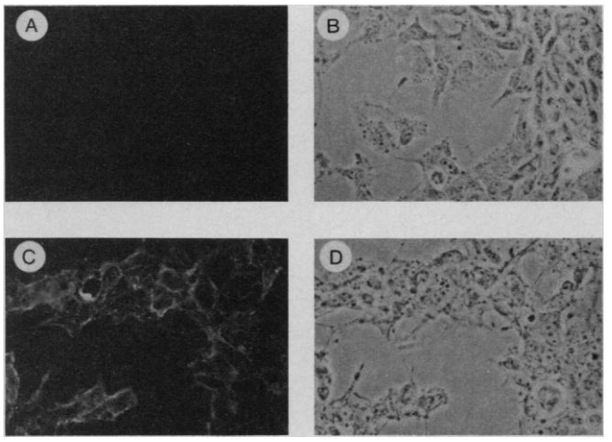

Fig. 1. Indirect immunofluorescence of vSIDK-infected cells by MAb 1C7. BSC-1 cells were infected with WR (**A** and **B**) or vSIDK (**C** and **D**) vaccinia virus, and indirect immunofluorescence was performed 48 hours later on live cells (A and C). Light microscopy of the same fields showed the resulting plaques (B and D).

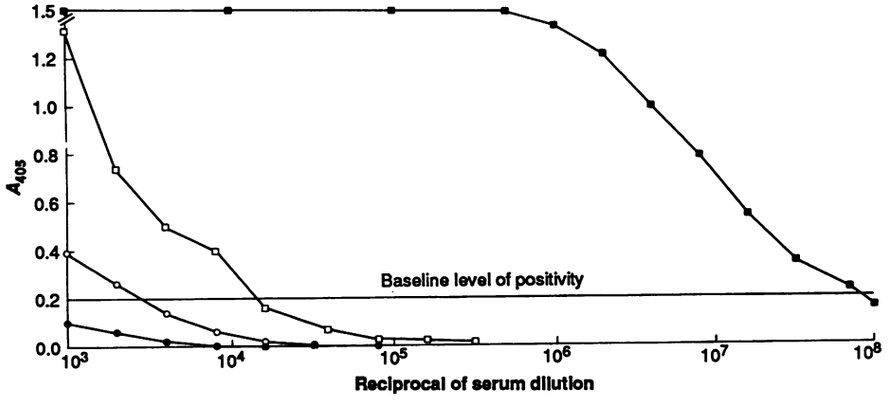

Fig. 2. After tertiary inoculation (open squares) by tail scratch with 10^7 pfu of vSIDK, ELISA titer of antibodies to Pfs25 increased fivefold over the primary immune response (open circles). Control antibody (closed circles) was below baseline positivity at a dilution of $1:10^3$, and a $1:10^8$ dilution of a 200 μg/ml solution of purified MAb 1D2 (closed squares) was required to reach baseline positivity.

To determine antibody titers to rPfs25 in mice inoculated with vSIDK, we developed an enzyme-linked immunosorbent assay (ELISA) with extracts of vSIDK-infected cells as the capture antigen. The optimal concentrations of capture antigen and a reliable method of determining the portion of the signal specific for antibodies to Pfs25 in immune sera were determined in pilot studies (*9, 10*). Purified MAb 1D2, a transmission blocking MAb similar to MAb 1C7 (*11*), could be detected by ELISA at a dilution greater than $1:10^8$ (Fig. 2). A concentration of MAb 1D2 of 200 μg/ml, which is equivalent to a 1:2 dilution in the ELISA (Fig. 2), was required for full transmission-blocking activity in membrane feeds (Table 1). After a single tail scratch inoculation with vSIDK, CAF-1 mice developed antibody titers to Pfs25 of 1:3000 by ELISA (Fig. 2). Sera from these CAF-1 mice failed to block transmission (*12*). When these mice were inoculated two more times with vSIDK virus, antibody titers to Pfs25 by ELISA rose fivefold to 1:16,000 (Fig. 2). At this titer, complete transmission-blocking activity in membrane feeds was achieved (Table 1), even when the immune sera were diluted 1:20 (*12*).

Studies have indicated that high (250 μg/ml) antibody titers to Pfs25 are required for complete transmission-blocking activity (*13*), titers that might be difficult to achieve and maintain in the field (*14*). The data presented here indicate that polyclonal immune sera of much lower titers, achieved by three inoculations with live attenuated

Table 1. Complete transmission-blocking activity after tertiary inoculation of CAF-1 mice with vSIDK. Sera or MAb 1D2 were mixed with *P. falciparum* gametocytes and fed to *Anopheles freeborni* by means of a membrane-feeding apparatus. Seven days after engorgement on the bloodmeal, mosquitoes were dissected, and their midguts were examined for *P. falciparum* oocysts (*11*). Sera from mice immunized three times by tail scratch with 10^7 plaque-forming units (pfu) of vSIDK were pooled. CAF-1 A is a pool of sera from mice 57 and 58. CAF-1 B is a pool of sera from mice 59, 60, and 61. The anti-WR is from a CAF-1 mouse inoculated three times with the WR strain of vaccinia virus. We calculated infectivity by dividing the mean oocyst count for each antiserum by the mean oocyst count for the control serum. The last column indicates the number of infected mosquitoes divided by the total number of mosquitoes dissected.

Antisera	Dilution or concentration	Mean oocyst (range)	Infectivity (% of control)	Infected/total
Normal human	1:2	9 (4–20)	100	16/16
anti-WR	1:2	9 (3–23)	100	21/21
MAb 1D2	1:2 (200 μg/ml)	0.1 (0–1)	1	2/19
MAb 1D2	1:4 (100 μg/ml)	1 (0–6)	11	5/11
MAb 1D2	1:8 (50 μg/ml)	1.4 (0–10)	16	7/11
MAb 1D2	1:16 (25 μg/ml)	3.5 (0–11)	39	12/25
CAF-1 A	Neat	0 (0)	0	0/26
CAF-1 A	1:2	0 (0)	0	0/16
CAF-1 B	Neat	0 (0)	0	0/22
CAF-1 B	1:2	0 (0)	0	0/20

Table 2. Transmission-blocking activity developed in six major histocompatibility complex (MHC)–disparate congenic mice after three inoculations with membrane extracts of vSIDK-infected cells. Control sera are pooled sera collected from all six congenic mouse strains after three inoculations with membrane extracts from WR strain–infected cells. Infectivity was calculated as in Table 1.

Antisera	Dilution or concentration	Mean oocyst (range)	Infectivity (% of control)	Infected/total
MAb 1D2	200 μg/ml	0 (0)	0	0/20
WR extract	1:2	4.5 (0–13)	100	14/20
C57B1/10	1:2	0.1 (0–1)	2	2/20
B10-A (3R)	1:2	0.15 (0–1)	3	3/20
B10-D2	1:2	0.1 (0–1)	2	2/20
B10-BR	1:2	0.2 (0–2)	4	3/20
B10-S (7R)	1:2	0.05 (0–2)	1	1/20
B10-S (9R)	1:2	0 (0)	0	0/20

virus, have complete transmission-blocking activity. The transmission-blocking activity titer of greater than 1:20 (*12*) in the polyclonal sera was at least tenfold higher than that of MAb 1D2, which was found to have a transmission-blocking titer of only 1:2 (Table 1), despite the polyclonal sera having at least a 1000-fold lower titer by ELISA than the MAb 1D2 (Fig. 2). Therefore, when compared to MAbs, polyclonal antibodies to recombinant Pfs25 appear to have qualitatively, and not just quantitatively, better transmission-blocking activity.

Good and co-workers (*15*) have shown that immunoresponsiveness to Pfs25 governed by *Ir* genes was nonrestricted in six different strains of H-2 congenic mice immunized with *P. falciparum* zygotes. To examine whether there was *Ir* gene–controlled nonresponsiveness to rPfs25 and to determine whether the ability to induce antibodies that had transmission-blocking activity was genetically restricted, we immunized the same six congenic mouse strains with either live vSIDK or membrane extracts from vSIDK-infected cells. After a single inoculation with live vSIDK, all six strains elicited a specific immune response to Pfs25 (Fig. 3) but did not block transmission (*12*). However, sera from congenic mouse strains immunized with three inoculations of membrane extracts from vSIDK-infected cells (*16*) all developed transmission-blocking activity (Table 2). These latter data indicate that the capacity to induce transmission-blocking antibodies to rPfs25 is not genetically restricted.

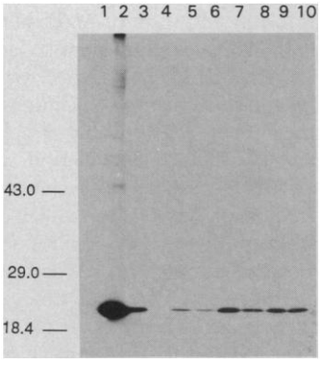

Fig. 3. Unrestricted recognition of Pfs25 from zygotes by sera to vSIDK produced in MHC-disparate congenic mouse strains. Serum from mice immunized by tail scratch with 10^7 pfu of WR strain (lane 4) or vSIDK (lanes 5 to 10) were pooled and used to immunoprecipitate Triton X-100 extracts of *P. falciparum* zygotes radiolabeled for 5 hours with [^{35}S]cysteine in M199 (*12*). Lanes: molecular size markers (lane 1; sizes indicated at left in kilodaltons); total antigen (lane 2); MAb 1C7 (lane 3); pool of all congenic mouse strains (lane 4); C57 BL/10 (lane 5); B10.S(7R) (lane 6); B10.BR (lane 7); B10.D2 (lane 8); B10.S(9R) (lane 9); and B10.A(3R) (lane 10).

Ideally, a transmission-blocking vaccine should induce high titer, long-lasting transmission-blocking antibodies after a single immunization. Subsequent natural infection would maintain or boost transmission-blocking activity. In this study, more than one inoculation with the live, attenuated, recombinant vaccinia virus was necessary to achieve high enough titers of antibodies to Pfs25 to block transmission. The low antibody titer observed in mice inoculated only once with recombinant virus may be due, in part, to the reduced replication of virus in mice (*17*) infected with a recombinant WR strain of vaccinia, in which the thymidine kinase gene has been disrupted. The subsequent inoculations quantitatively boosted the titer fivefold, as measured by ELISA, but also qualitatively changed the antibody, as assayed by transmission-blocking activity. The tertiary immunization sera developed transmission-blocking activity that was retained beyond a fivefold dilution. These data suggest that after boosting antibodies to new epitopes were induced, or immunoglobulin class switching or somatic mutation occurred to produce a more effective transmission-blocking antibody. The immunogenicity of Pfs25 in humans and the question of whether a natural infection, after a primary inoculation with recombinant virus, will boost antibodies titers to rPfs25 to levels that will block transmission remain to be determined.

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10. Briefly, HeLa cells were infected at a multiplicity of infection of 1 to 2 with either the vSIDK or the WR strain of vaccinia virus. After 48 hours, the cells were harvested by low-speed centrifugation and the cell pellet was resuspended in two and one-half volumes of swelling buffer (10 mM tris, pH 9.0). The cells were disrupted by three cycles of freeze-thaw in ethanol–dry ice, followed by sonication. We first fractionated the cell lysate by allowing the cellular debris to settle out of suspension for 2 to 3 hours on ice. The portion still in suspension was further fractionated by centrifugation at 5500*g* for 10 min at 4°C into membrane-bound (pellet) and soluble fractions (supernatant). The membrane-bound fraction was resuspended in coating buffer (15 mM sodium carbonate, 35 mM sodium bicarbonate, 0.02% w/v sodium azide, pH 9.6) to a final concentration of total protein of 10 to 20 μg/ml. Membrane suspension (100 μl) was added to each well of a polystyrene microtiter plate (Immulon 1, Dynatech Labs) and incubated at 4°C for 16 hours. The wells were subsequently blocked with 1% bovine serum albumin in coating buffer. Test serum or MAb was then added to each well at various dilutions and incubated at 37°C for 90 min. After thorough washing with 0.5% Tween 20 in phosphate-buffered saline (TPBS), a 1:1000 dilution of goat antibodies to mouse immunoglobulin G conjugated to alkaline phosphate (Jackson ImmunoResearch Laboratories) was added to each well and incubated at 37°C for 90 min. The wells were thoroughly washed with TPBS, and then substrate was added and incubated at 37°C for 15 min. Absorbance (A) was read at 405 nm. Each well with vSIDK membrane extract had a matched well with WR membrane extract. The amount of extract added to each well was adjusted such that matched wells had identical A_{405} when mouse serum to WR (anti-WR) was used as the primary antibody. We could, therefore, determine the signal specific for Pfs25 by subtracting the A_{405} of the well with the WR membrane extract from the A_{405} of the well with the matched vSIDK membrane extract. When calculated in this manner, a baseline level of positivity was defined as an A_{405} of 0.20.
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16. Membrane extracts prepared above (*9*) were treated with psoralen at a final concentration of 5 μg/ml for 10 min at room temperature, and the cellular debris was allowed to settle at 4°C for 2 hours. To completely inactivate any live virus, we irradiated the supernatant with long-wave (365 nm) ultraviolet at a distance of 10 cm for 4 min with gentle agitation. A 100-μl aliquot of this preparation was found to have no lytic activity on a monolayer of BSC-1 cells.

A 1-ml aliquot of the psoralen-treated stock (1 mg/ml) was diluted in 6 ml of PBS. Two milliliters of the diluted stock were added to the Ribi adjuvant system (Ribi ImmunoChem Research), in accordance with the manufacturer's specifications, and 0.2 ml were administered into the peritoneum. Mice were given booster inoculations on days 21 and 48.
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Two- Rather Than Three-Dimensional Representation of Saccades in Monkey Superior Colliculus

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Saccades are controlled by neurons in the brainstem reticular formation that receive input from the superior colliculus and cortex. Recently two quantitative models have been proposed for the role of the colliculus in the generation of three-dimensional eye movements. In order to test these models, three-dimensional eye movements were measured in the alert monkey to investigate whether the saccadic motor map of the superior colliculus is two-dimensional, representing retinal target vectors, or three-dimensional, representing three-dimensional motor error for the rotation of the eye. Electrical stimulation of the superior colliculus produced two-dimensional, not three-dimensional, eye movements. It is therefore concluded that the collicular motor map is two-dimensional.

THE MONKEY SUPERIOR COLLICULUS (SC) is a brainstem structure that is important in the generation of saccadic eye movements (*1*). Electrical stimulation of the SC produces saccades with short latencies at low stimulation thresholds. Saccade amplitude and direction depend predominantly on the site of stimulation (*2, 3*). Cells in the deeper layers of the SC burst vigorously for saccades that are directed into their movement field (the range of saccade vectors for which a particular SC cell is activated), and the neural activity related to movement is tightly coupled to saccade onset (*1, 4*). Furthermore, the SC provides an important input to the reticular formation, where the burst generator for all rapid eye movements is situated (*5, 6*). Whereas ablation of the SC (*7, 8*) causes only minor permanent deficits in saccadic performance, small local injections of either muscimol or lidocaine (*9*), which inhibit neural activity, cause profound deficits for saccades directed into the affected movement field. Parallel pathways, presumably incorporating the frontal eye field (FEF), may be able to compensate for most of the deficits after ablation of the SC (*7*).

So far, researchers have investigated the function of the SC by measuring eye movements in two dimensions (horizontal and vertical). Recently, however, theoretical studies have shown that a complete description of the rotational kinematics of eye movements must be in three dimensions, that is, it must include torsion (*10, 11*). There are two ways of describing the kinematics of saccades. One description is a trajectory; eye position is described by a virtual rotation from a head-fixed reference position (primary position). Experimental evidence shows that all virtual rotation axes describing eye positions lie in a single plane [Listing's law (*12*)], which is defined as Listing's plane (Fig. 1) if the head is upright and stationary. The other description uses the rotation of the eye from the starting position of the saccade to the instantaneous position. It has been shown experimentally that in this description saccades have fixed angular velocity axes that are not confined to a plane (*13*) and therefore require a three-dimensional (3-D) parameter space. These two descriptions are equivalent and are a consequence of the noncommutativity of rotations in 3-D space (*10, 11*).

We have investigated the neural implementation of Listing's law. Listing's law may be implemented upstream from the motor SC [the quaternion model (*11*)]. The axis, when the eye moves from initial position $\mathbf{r}_A$ to final position $\mathbf{r}_B$, both in Listing's plane (Fig. 1), is thought to be coded in the motor colliculus as the rotation vector (*10, 14, 15*):

$$\mathbf{r}_{AB} \approx \mathbf{r}_B - \mathbf{r}_A + \mathbf{r}_A \times \mathbf{r}_B \qquad (1)$$

where $\times$ denotes the vector outer product (*10, 11*). In this model, the collicular vector $\mathbf{r}_{AB}$, the direction of which is the angular velocity axis of an eye rotation, will, in general, be tilted out of Listing's plane because its torsional component, $r^x_{AB} = (\mathbf{r}_A \times \mathbf{r}_B)^x$, is nonzero whenever $\mathbf{r}_A$ and $\mathbf{r}_B$ are nonparallel rotation vectors (*13*).

A corollary of Eq. 1 is that electrical stimulation will, in general, yield saccades that bring the eye out of Listing's plane (*11*) in a specific way: the torsional component of the eye, achieved after stimulation, is determined by

$$r^x_S \approx \|\mathbf{r}_{AB}\| \cdot r^{\perp} \qquad (2)$$

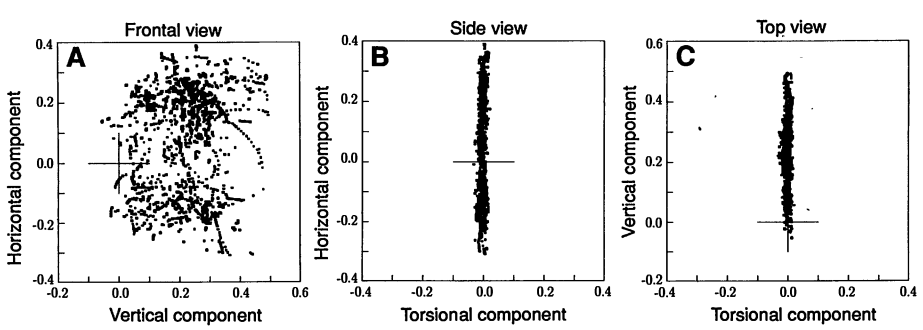

Fig. 1. Listing's law for eye position of visually evoked eye movements. Eye positions (*15*) were sampled at regular intervals during and between saccades in the light (1575 data points) with the head pitched downward by 15°. All eye positions lie in a well-defined plane with zero torsion. Typical standard deviation in the torsional direction was less than 1°. The plane is perpendicular to the torsional direction, thus defining the direction of primary position (cross mark). Data from monkey Ca. (**A**) Frontal (horizontal-vertical) view of Listing's plane. The center of the oculomotor range is, for this monkey, downward from primary position. (**B**) Side view of Listing's plane along the interaural line. (**C**) Top view of Listing's plane along the vertical axis. Units for both figures are the following: a rotation vector component of 0.1 corresponds approximately to $\rho = 10°$ according to the formula $\mathbf{r} = \tan(\rho/2) \cdot \mathbf{n}$ (*14, 15*).

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