after the empathy induction and in selfish types [motive induction × individual type, F(1,30) = 4.9, P = 0.034] (Fig. 4C).

Motives are purely mental constructs that are not directly observable. Here we show, however, that distinct motives have a distinct neurophysiological representation in the brain. Although the empathy and the reciprocity motive increase the frequency of altruistic acts by the same amount relative to the baseline condition, they are associated with different patterns of brain connectivity that enabled us to predict them. Our results indicate that there is no high accuracy, we predicted each subject’s induced motive with a classifier whose parameters were not influenced by that subject’s brain data (nor by that subject’s behavioral data). Instead, the parameters of the classifier were solely informed by other subjects’ brain data. This means that the motive-specific brain connectivity patterns are generalizable across subjects. The distinct and across-subject-generalizable neural representation of the different motives thus provides evidence for a distinct neurophysiological existence of motives.

The findings also provide mechanistic insights into the neural underpinnings of important altruistic motives and how motive inductions change the underlying neural network. In particular, predominantly selfish individuals were characterized by a low or even negative connectivity from ACC→AI in the baseline condition, whereas predominantly prosocial individuals displayed a positive connectivity between these regions. However, when we induce the empathy motive, the selfish, but not the prosocial, types become more altruistic and show a substantial increase in ACC→AI connectivity. Thus, after the empathy induction, selfish individuals resemble “homegrown” unconditional altruists in terms of both brain connectivity and altruistic behavior. This contrasts with the effect of inducing the reciprocity motive, which renders the prosocial, but not the selfish, types more altruistic and increases only the prosocial type’s AI→VS connectivity.

We obtain these mechanistic insights because the inputs into the support vector machine are not merely brain activations but small brain models of how relevant brain regions interact with each other (i.e., functional neural architectures). Thus, by correctly predicting the induced motives, we simultaneously determine those mechanistic models of brain interaction that best predict the motivation. It is these models that deliver the mechanistic insights into brain function and how changes in brain function relate to behavioral changes due to motive inductions. Our study, therefore, also demonstrates how “mere prediction” and “insights into the mechanisms” that underlie psychological concepts (such as motives) can be simultaneously achieved if functional neural architectures are the inputs for the prediction.

EBOLA VIRUS

Isolation of potent neutralizing antibodies from a survivor of the 2014 Ebola virus outbreak

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Antibodies targeting the Ebola virus surface glycoprotein (EBOV GP) are implicated in protection against lethal disease, but the characteristics of the human antibody response to EBOV GP remain poorly understood. We isolated and characterized 349 GP-specific monoclonal antibodies (mAbs) from the peripheral B cells of a convalescent donor who survived the 2014 EBOV Zaire outbreak. Remarkably, 77% of the mAbs neutralize live EBOV, and several mAbs exhibit unprecedented potency. Structures of selected mAbs in complex with GP reveal a site of vulnerability located in the GP stalk region proximal to the viral membrane. Neutralizing antibodies targeting this site show potent therapeutic efficacy against lethal EBOV challenge in mice. The results provide a framework for the design of new EBOV vaccine candidates and immunotherapies.

In recent years, Ebola virus (EBOV) outbreaks have increased in frequency, duration, and geographical spread, underscoring the need for pre- and post-exposure treatments (2). The membrane-anchored EBOV glycoprotein (GP) trimer is the sole known target for protective antibodies and is currently the primary target for antiviral vaccines and therapies. (2, 3) A small number of protective monoclonal antibodies (mAbs) to GP have been isolated from immunized mice.
and recent structures of these antibodies in complex with GP have illuminated key sites of vulnerability on the EBOV glycoprotein (3–7). However, only a small number of mAbs to GP have been isolated from human EBOV survivors (8–10), and therefore the characteristics of the human antibody response to EBOV GP remain largely undefined.

In this study, we aimed to comprehensively profile the human B cell response to EBOV GP by cloning an extensive panel of mAbs to GP from the peripheral B cells of a convalescent donor (subject 45) who survived the 2014 EBOV Zaire outbreak. Three months after primary infection, the donor plasma showed strong immunoglobulin G (IgG) binding reactivity to EBOV GP and potent neutralizing activity, suggesting that this donor had mounted a robust neutralizing antibody (Nab) response to GP by this time point (fig. S1, A and B). To assess the magnitude of the B cell response to EBOV GP, B cells were stained with a fluorescently labeled EBOV GP ectodomain (GPATM) (4) and analyzed by flow cytometry. Approximately 3% of IgG+ B cells were specific for GPATM (fig. S2), which is comparable to the percentage of circulating antigen-specific peripheral B cells observed during chronic HIV infection and after primary dengue infection (11, 12). Cognate antibody heavy- and light-chain pairs were rescued from 420 individual GPATM-reactive B cells by single-cell polymerase chain reaction (PCR) cloning and expressed as full-length IgGs in an engineered strain of Saccharomyces cerevisiae (13). Of the 420 cloned mAbs, 349 bound to EBOV GP in preliminary binding screens (table S1). Analysis of the heavy- and light-chain variable regions (VH andVk, respectively) revealed that the anti-GP repertoire was highly diverse, containing 294 independent clonal lineages (fig. S3A and table S2). This result contrasts with previously described anti-HIV and anti-influenza repertoires, which show a significantly higher degree of clonal restriction (11, 14). Comparison to non-GP-reactive antibodies (15) revealed that the EBOV GP-specific repertoire was skewed toward Ig light-chain kappa (Igk) versus Ig light-chain lambda (Igl) and longer heavy-chain complementarity-determining region 3 (CDRH3) lengths (fig. S3, B and C, and table S2). Similar biases have also been observed in HIV-1-infected patient repertoires (11, 12). VH and Vk germline gene usage in the GP-specific repertoire was similar to non-GP-specific repertoires (15, 16) (fig. S3, D and E, and table S2). As expected for antibodies derived from IgG+ B cells, almost all of the GP-specific clones were somatically mutated, with an average of 5.1 and 2.7 nucleotide substitutions in VH and Vk, respectively (fig. S3F and table S2).

To map the antigenic specificities of the mAbs to GP, we produced 321 IgGs in larger quantities and performed biolayer interferometry (BLI) binding experiments with several GP variants. We first tested binding to EBOV GPATM and a mucin-like domain (Fig. 1A and table S3). About 30% of the mAbs showed increased binding responses and faster association rates to GPΔmuc as compared to GPATM (fig. S4), suggesting that these mAbs probably recognize epitopes that are partially occluded by the mucin-like domain. We next tested the mAbs for binding to a secreted GP isof orm, sGP, which is expressed as a disulfide-linked GPI dimer containing the majority of the mucin-like domain-free GPI core and glycan cap sequence (fig. S5) (17, 18). This analysis revealed that 39% of GPΔmuc-reactive mAbs failed to bind to sGP, 2% bound with similar apparent affinity to both GPΔmuc and sGP, and 59% reacted with both proteins but bound with higher apparent affinity to sGP (Fig. 1, B and C, and table S3). The latter result is consistent with previous studies showing that sGP is secreted in large quantities during natural infection and may behave as an antigenic decoy by redirecting the immune response toward epitopes that are either inaccessible on surface GP or shared between the two proteins (17, 19).

To further define the epitopes targeted by the mAbs to GP, we performed competitive binding experiments (20). We first tested the 321 mAbs for competition with two well-characterized murine mAbs, 1H3 and 13C6, that recognize overlapping epitopes in the glycan cap (4). The vast majority of sGP cross-reactive binders competed with one or both mAbs, suggesting that they also bind within the glycan cap (Fig. 2A and fig. S6A). We next tested the GP-specific mAbs for competition with KZ52, a human antibody that binds at the interface of GP1 and GP2 (6, 8). Approximately half of the GP-specific binders competed with KZ52 (Fig. 2A and fig. S6B), suggesting that this antigenic site is a common target for antibodies elicited by natural EBOV infection, at least for the donor studied. Because KZ52 has been shown to exhibit specificity for Zaire GP (6), we next tested selected KZ52 competitors for cross-reactivity with Sudan (SUDV) GP and Bundibugyo (BDBV) GP. Similar to KZ52, most of these mAbs did not show broad species cross-reactivity (Fig. 2B and fig. STA). However, in contrast to KZ52 and other well-characterized GP base binders (4), most of the KZ52 competitor mAbs failed to react with a minimal thermodenatured cross-reactive core, in which both the mucin domain and glycan cap regions had been proteolytically removed (GPΔmuc) (4) (fig. 2C). Thus, this class of antibodies appears to target specific epitopes that either directly overlap with the KZ52 epitope or are sterically inhibited...
Fig. 2. Epitope mapping. (A) Percentage of sGP-reactive and sGP–non-reactive mAbs directed against each antigenic site on EBOV GP. Epitope binning was performed using a previously described yeast-based competition assay (20). (B) Percentage of selected KZ52 competitors that cross-react with SUDV GP and BDENV GP. Binding cross-reactivity was assessed by enzyme-linked immunosorbent assay (ELISA). (C) ELISA binding of selected KZ52 competitors to a minimal GP core that contains deletions in the mucin-like domain and glycan cap (GPCL). ELISA binding is expressed as the optical density at 405 nm (OD405) reading at a concentration of 0.2 μg/ml. (D) Percentage of selected KZ52 noncompetitors that cross-react with SUDV GP and BDENV GP. Binding cross-reactivity was assessed by ELISA. (E) Summary of the antigenic sites targeted by the mAbs to GP. All data are representative of two or more independent experiments.

Fig. 3. Neutralizing activity of mAbs to GP. (A) Percentage of mAbs in each competition group that reached PRNT50 or PRNT80 at concentrations ≤50 μg/ml. The total number of mAbs tested from each competition group is shown at the top of the corresponding bar. (B) PRNT50 and PRNT80 values of selected mAbs from each competition group. KZ52 IgG is included for comparison (green inverted triangles). Red bars indicate median PRNT50 and PRNT80 values. Neutralization assays were performed using a live virus plaque reduction assay. PRNT50 and PRNT80 values represent the concentration of IgG required to reduce viral infectivity by 50 and 80%, respectively. All data are representative of two independent experiments.
We next measured the neutralizing activity of the B cell–derived mAbs using a live virus plaque reduction neutralization (PRNT) assay. Because of the large number of mAbs and the high-throughput nature of our study, initial neutralization screening was performed using a single concentration of purified IgG (table S5 and fig. S9). Remarkably, 77 and 63% of the mAbs reduced viral infectivity by 50 and 80% (PRNT50 and PRNT80, respectively, at concentrations ≤50 μg/ml (Fig. 3A and table S5). Control experiments with yeast-produced and CHO-produced (CHO, Chinese hamster ovary cells) IgGs demonstrated that functional activity is probably not affected by the host production system (fig. S8). Analysis of neutralizing activity by a competition group revealed that the majority of competition groups contained a proportion of NAbs, with the KZ52 and 13C6/1H3 competition groups containing the highest proportion of NAbs (Fig. 3A and table S5). The latter result was unexpected, because 13C6 and 1H3 only weakly neutralize in the absence of complement (7, 21). We next performed neutralization titration experiments in order to evaluate neutralization potency. These results showed that several NAbs, particularly those in the ADI-15974 and KZ52 competition groups, exhibited extraordinary potency. Half of the NAbs tested from the ADI-15974 competition group, and two of the NAbs tested from the KZ52 competition group, neutralized with PRNT50 values ≤0.05 μg/ml (Fig. 3B and table S5). In contrast, the majority of 13C6 and/or 1H3 competitor mAbs neutralized with relatively modest potency, with PRNT50 values averaging 3.3 μg/ml. We conclude that the GP-specific antibody repertoire in the donor studied contains a high proportion of NAbs, the most potent of which bind to epitopes overlapping those of KZ52 or ADI-15974.

To structurally define the epitopes recognized by the NAbs, we used single-particle electron microscopy (EM) to examine five potent NAbs, representing each of the four major competition groups, in complex with fully glycosylated EBOV GPATM. These NAbs included ADI-15731 (a 13C6 competitor), ADI-15734 and ADI-15762 (KZ52 competitors), ADI-15758 (an ADI-15974 competitor), and ADI-15859 (an ADI-15810 competitor). We were able to obtain negative-stain two-dimensional (2D) class averages for all five complexes of Fabs bound to EBOV GPATM (fig. S10) and 3D reconstructions for four of the Fab:EBOV GPATM complexes at 18 to 24 Å resolution (Fig. 4 and fig. S11). In agreement with the competitive binding data, the EM reconstruction of ADI-15731 showed that this NAb binds within the glycan cap, with a footprint approximately between the epitopes recognized by 13C6 and 1H3 and with a similar angle of approach (Fig. 4 and fig. S12A). We next examined the two KZ52 competing NAbs, ADI-15734 and ADI-15762. As anticipated, ADI-15734 bound to EBOV GPATM at the GP1/GP2 interface, slightly adjacent to the KZ52 epitope and at a similar angle of approach (Fig. 4 and fig. S12C). In contrast, ADI-15762 actually binds within the glycan cap, but with a shallow binding angle that probably sterically occludes the KZ52 epitope (Fig. 4 and fig. S12B). Last, we determined the structure of EBOV GPATM in complex with ADI-15758 (an ADI-15974 competitor) on the left. One of the most potent NAbs described in this panel. The EM reconstruction shows that ADI-15758 binds to a region proximal to the viral membrane, distal to all previously described epitopes, and below the body of the trimeric EBOV GP structure (Fig. 4 and fig. S12D). Although this region has not yet been structurally characterized at high resolution in the pre-fusion GP context, it corresponds to the α-helical heptad repeat 2 (HR2; residues 613 to 637) defined in the post-fusion conformation. Docking of the EBOV GP crystal structure into the reconstruction suggests that the ADI-15758 epitope is within the C-terminal 24 residues of GP2 contained in the EBOV GPATM construct (6). Three Fab molecules...
could be visualized in the 2D class averages (fig. S10), suggesting that the HR2 region may exist as a three-helix bundle in the pre-fusion GP structure (6). Additionally, although we were not able to generate a 3D reconstruction of ADI-15859 (an ADI-15810 competitor) bound to EBOV GPA, the negative-stain 2D class averages indicate that this mAb also binds within the GP stalk region. Collectively, these data suggest that the GP stalk region containing the HR2 helices is an accessible antigenic region targeted by NAbs, a proportion of which exhibit remarkable neutralization potency.

Finally, we sought to determine whether certain NAbs to GP showed greater in vivo efficacy than others. For this experiment, several NAbs were selected from each competition group and evaluated for post-exposure therapeutic efficacy against lethal EBOV challenge in a murine infection model (23). Groups of mice were challenged with a target dose of 100 plaque-forming units (PFU) of mouse-adapted EBOV (MA-EBOV), followed by a single 100 μg dose of mAb at 2 days post-infection (dpi). The previously described neutralizing mAb 2G4, a component of the ZMapp cocktail, was also included for comparison (3). Of significance, all of the ADI-15974 competitor NAbs (GP stalk binders) provided significant post-exposure protection, with survival rates ranging from 60 to 100% and average weight loss ranging between 8 and 10% (Fig. 5A, fig. S13, and table S6). Five out of the six NAbs in the KZ52 competition group were also highly effective in protection, with survival rates ranging between 60 and 100% (Fig. 5B and table S6). With the exception of ADI-15818 (a KZ52 competitor), all of the NAbs in these two competition groups showed greater therapeutic efficacy than 2G4, which only provided 40% protection under these conditions. In contrast to the ADI-15974 and KZ52 competitor NAbs, the NAbs targeting the glycan cap (13C6/1H3 competitors) and undefined epitopes generally showed little to no therapeutic efficacy (Fig. 5, C and D, fig. S13, and table S6). Only ADI-16037 (a 13C6 competitor NAb) provided potent protection, yielding 80% survival and 7% average weight loss. The remaining NAbs in these groups yielded ≤50% survival, which in most cases was not a statistically significant increase in protection over the negative control (Fig. 5, C and D, and table S6). This result is consistent with previous studies showing that mAbs targeting the glycan cap generally do not afford significant protection when administered at 1 to 2 dpi (3, 24). In summary, most of the NAbs targeting the GP stalk region (ADI-15974 competitors) and the GP1/GP2 interface (KZ52 competitors) provided significant post-exposure protection against lethal EBOV challenge, whereas NAbs targeting the glycan cap (13C6/1H3 competitors) and undefined regions generally showed little to no therapeutic efficacy under these conditions.

We have shown that the human B cell response to EBOV GP is composed of a broad diversity of clones that primarily target three non-overlapping antigenic sites on the GP spike: the glycan cap; the GP1/GP2 interface; and the stalk, inclusive of the HR2 helices (fig. S14). A substantial fraction of the mAbs cloned from GP-specific B cells show neutralizing activity, demonstrating that at least in this donor, NAb responses can develop relatively early after EBOV infection. The most potentially neutralizing and therapeutically effective mAbs in our panel target the GP1/GP2 interface and the GP stalk region, suggesting that these epitopes may be promising targets for rational vaccine design. In addition, the observation that EBOV escape variants can emerge after treatment with the MB-003 antibody cocktail highlights the need for protective mAbs that target new antigenic sites, such as those described here targeting the GP stalk (25, 26).

### References and Notes

12. M. Beltramello et al., Cell Host Microbe 6, 271–283 (2010).
IMMUNOGENOMICS

Regulatory evolution of innate immunity through co-option of endogenous retroviruses

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Endogenous retroviruses (ERVs) are abundant in mammalian genomes and contain sequences modulating transcription. The impact of ERV propagation on the evolution of gene regulation remains poorly understood. We found that ERVs have shaped the evolution of a transcriptional network underlying the interferon (IFN) response, a major branch of innate immunity, and that lineage-specific ERVs have dispersed numerous IFN-inducible enhancers independently in diverse mammalian genomes. CRISP-R7Cas9 depletion of a subset of these ERV elements in the human genome impaired expression of adjacent IFN-induced genes and revealed their involvement in the regulation of essential immune functions, including activation of the AIM2 inflammasome. Although these regulatory sequences likely arose in ancient viruses, they now constitute a dynamic reservoir of IFN-inducible enhancers fueling genetic innovation in mammalian immune defenses.

Changes in gene regulatory networks underlie many biological adaptations, but the mechanisms promoting their emergence are not well understood. Transposable elements (TEs), including endogenous retroviruses (ERVs), have been proposed to facilitate regulatory network evolution because they contain regulatory elements that can amplify in number and/or move throughout the genome (1–3). Genomic studies support this model (4), revealing that a substantial fraction of TE-derived noncoding sequences evolve under selective constraint (3, 5), are frequently bound by transcription factors (6–10), and often exhibit cell type–specific chromatin states consistent with regulatory activity (11, 12). These observations implicate TEs as a potential source of lineage-specific cis-elements capable of rewiring regulatory networks, but the adaptive consequences of this process for specific physiological functions remain largely unexplored.

We investigated the evolution of gene regulatory networks induced by the proinflammatory cytokine interferon-γ (IFNG). Interferons are proinflammatory signaling molecules that are released upon infection to promote transcription of innate immune factors, collectively defined as IFN-stimulated genes (ISGs) (13). ISGs are regulated by cis-regulatory elements that are bound by IFN (interferon regulatory factor) and STAT (signal transducer and activator of transcription) transcription factors upon activation of IFN signaling pathways (13). Although innate immune signaling pathways are conserved among mammals, the transcriptional outputs of these pathways differ across species (14, 15), likely reflecting lineage-specific adaptation in response to independent host-pathogen conflicts. Thus, these pathways provide useful systems that allow us to investigate whether TE-derived regulatory elements influence biological outcomes.

To explore the influence of TEs on IFNG-inducible regulatory networks, we examined their contribution to IFI151 and STAT1 binding sites with the use of published chromatin immunoprecipitation sequencing (ChIP-seq) data for three human cell lines treated with IFNG: K562 myeloid-derived cells, HeLa epithelial-derived cells, and primary CD14+ macrophages (16, 17). Our initial analysis revealed 27 TE families enriched within IFNG-induced binding peaks in at least one of the data sets examined (18) (table S1 and fig. S1, A and B) and included TEs previously predicted to be cis-regulatory elements (11, 19). These sequences contained evolutionarily young to ancient TE families, of which the majority (20 of 27) originated from long terminal repeat (LTR) primate regions of ERVs (Fig. 1A). These data suggest that ERVs, which arose from ancient retroviral infections and currently constitute 8% of the human genome (20), represent a source of novel binding sites bound by IFNG-inducible transcription factors.

We next investigated whether these ERVs may contribute to IFNG-inducible regulation of adjacent cellular genes. ERVs bound by STAT1 and/or IFI151 in CD14+ macrophages were strongly enriched near ISGs (binomial test, P = 1.4 × 10–22; Fig. 1B and fig. S2), determined from a matched RNA-seq data set (table S2) (18, 21). A complementary approach using the genomic regions enrichment of annotations tool (GREAT) (22) revealed enrichment of CD14+ STAT1-bound and/or IFI151-bound ERVs near genes annotated with immune functions (fig. S3, A and B). These findings suggest a potentially widespread role for ERVs in the regulation of the human IFNG response.

MER41 is an endogenized gammaretrovirus that invaded the genome of an anthropoid primate ancestor ~45 to 60 million years ago with 7190 LTR elements, from six subfamilies (MER41A, B, C, D, E, and G), now fixed in the human genome (fig. S4A). Our analysis revealed the primate-specific MER41 family of ERVs as a source of IFNG-inducible binding sites (fig. S4B), with nearly 1000 copies in humans (N = 962) bound by
Isolation of potent neutralizing antibodies from a survivor of the 2014 Ebola virus outbreak


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Profiling the antibody response to Ebola

The recent Ebola virus outbreak in West Africa illustrates the need not only for a vaccine but for potential therapies, too. One promising therapy is monoclonal antibodies that target Ebola’s membrane-anchored glycoprotein (GP). Bornholdt et al. isolated and characterized 349 antibodies from a survivor of the 2014 outbreak. A large fraction showed some neutralizing activity and several were quite potent. Structural analysis revealed an important site of vulnerability on the membrane stalk region of GP. Antibodies targeting this area were therapeutically effective in Ebola virus–infected mice.

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