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ORIGINAL ARTICLE

Fever as an evolutionary agent to select immune complexes interfaces

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Abstract

We herein analyzed all available protein–protein interfaces of the immune complexes from the Protein Data Bank whose antigens belong to pathogens or cancers that are modulated by fever in mammalian hosts. We also included, for comparison, protein interfaces from immune complexes that are not significantly modulated by the fever response. We highlight the distribution of amino acids at these viral, bacterial, protozoan and cancer epitopes, and at their corresponding paratopes that belong strictly to monoclonal antibodies. We identify the "hotspots", i.e. residues that are highly connected at such interfaces, and assess the structural, kinetic and thermodynamic parameters responsible for complex formation. We argue for an evolutionary pressure for the types of residues at these protein interfaces that may explain the role of fever as a selective force for optimizing antibody binding to antigens.

Keywords Fever · Immune complexes · Binding interfaces · Binding affinity · mAb

Introduction

Formation of macromolecular complexes between proteins is a central feature of cellular processes (Peleg et al. 2014). While essential for cell metabolism and replication, it is recognized that many of these associations can be non-specific or prone to aggregation (Deeds et al. 2007). Promiscuous protein binding may occur within the extremely crowded cellular environment (Levy et al. 2012), in a haphazard manner that depends on their concentration, net charge and structural surface properties (Mu et al. 2017). As such, the evolutionary pressure towards designing specific protein interactions encompasses both their expression levels and their chemical properties. Protein–protein interfaces differ in their characteristics and amino acid composition from the rest of the protein surface (Keskin et al. 2008). For example, large hydrophobic and uncharged polar residues are more frequently encountered in the interfaces of hetero-complexes, compared to the rest of the surface (Jones and Thornton 1997), whereas charged residues are more frequent on the exposed, non-interface surface. This distinction is manifest in both intracellular and extracellular proteins, and most cellular functions that proteins possess are due in large part to the residues exposed to the solvent that are also evolutionarily more conserved (Schreiber 2020). Such molecular specificity of protein interactions, both in terms of unique partnerships and their highly ordered three-dimensional orientation, is key for information processing and ensures adaptation to the environment, including to the presence of pathogens (Yan et al. 2008; Jayashree et al. 2019).

An important aspect of adaptive immunity is the antigen-specific lymphocyte response, in particular with the B-cell antibodies (Ab) involved, among others, in opsonization and neutralization of bacteria and viruses. Fundamental to these immune responses is the formation of antigen–antibody complexes, where the antigen (Ag) binding sites dictate what epitopes will be recognized by the Ab paratopes and vice-versa. Within Ab, the N-terminal variable domains of the light chain (VL) and of the heavy chain (VH) associate non-covalently into the Fv-fragment. Three hyper-variable regions (complementarity determining regions or CDR) in

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VL and VH account for about 25% of the variable regions and contain the amino acids directly interacting with Ag (Mian et al. 1991). The CDR amino acid composition differs from the residue composition in other Ab loops and also from each other (Collis et al. 2003; Zhao and Li 2010; Raghunathan et al. 2012). As an adaptive measure to the presence of an ever changing landscape of molecular epitopes, germline encoded antibodies progressively mature their binding affinity and specificity for various target antigens by changing the residue composition of their CDR.

Higher binding affinity for antigens is achieved by fast association rates in conjunction with slow dissociation rates, in a process directly influenced, among other factors, by temperature. In most cell-based assays, however, the affinity measurements of antibody interactions are typically performed at room temperature (20–25 °C) or at 4–8 °C, without explicitly considering the potential impact of the physiological temperature. Indeed, when assays are performed at 37 °C on living cells and in real time, affinity of monoclonal antibodies (mAb) binding increases by almost an order of magnitude compared to measurements at standard 25 °C temperature (Encarnação et al. 2017). The thermal optimum of antigen–antibody complex formation depends both on the precise chemical nature of the epitope and paratope, and on the type of bonds formed at different temperatures (Ragone 2001). Temperature has a modulatory role in optimizing interfaces of immune complexes, and variations in core body temperature are known to have a key beneficial influence on infectious diseases' outcome (Kluger 1979). Even in the organisms that are unable to induce fever, studies showed that survival of e.g. *Dipsosaurus dorsalis* iguana is increased if the animal is allowed to behaviorally raise its core temperature by only 2 °C, after infection with the Gram-negative bacterium *Aeromonas hydrophila* (Vaughn et al. 1974). Because producing fever comes at a cost of approximately 10% of basal metabolism to generate 1 °C increase in core body temperature, physiological fever is a highly regulated and reversible process (Toft and Stroem 2018). Physiological fever, as opposed to pathological fever, is thus a temporary increase in body temperature that is relevant for pathogen clearance at multiple levels (Evans et al. 2015). These may include, for instance, prevention of pathogen replication or shedding (Yamaya et al. 2019). Indeed, there is a need to go beyond the 37 °C threshold and study immune complexes at febrile temperatures relevant for the disease under study. In the rare instances where immune complexes are studied in silico and in vitro at fever temperatures, important conclusions have been drawn as to the clinical outcomes following infections. For the current pandemic, SARS-CoV-2 spike protein has been shown to bind the ACE2 receptor with lower affinity at 40 °C vs. 37 °C (Zhou et al. 2021), while the spike protein itself is subject to temperature-dependent motions that expose new epitopes through hinge movements (Rath and Kumar 2020). Compared to the human physiological temperature, *Plasmodium falciparum* decreases its attachment to red blood cells at 41 °C (Lim et al. 2020). Similarly, *Salmonella enterica* serovars Typhi, Paratyphi A and Sendai markedly attenuate their motility and epithelial cell invasion at 39–42 °C (Elhadad et al. 2015). Recent in vitro results indicate that febrile temperatures impair hemolysis in hemolytic strains of *Escherichia coli* and *Staphylococcus aureus* (Palela et al. 2022). As protein flexibility, the "fourth dimension" of protein structure (Fragai et al. 2006), is directly affected by temperature, it is imperative that the role of pathologically relevant temperatures be further investigated. In this work, we delineate and describe the residues present at the interfaces formed by immune complexes that are elicited by fever-inducing pathogens or relevant carcinoma and blood cancers. We propose that fever may influence the activity and the mutual affinity between each protein partner in some immune complexes. As such, optimization of the binding interfaces with respect to temperature, involving both the paratope and epitope, may be a selective agent ensuring: (1) exposure of new epitopes and (2) enhanced, thermally assisted antibody binding. Parsing out the specific enrichment in certain residues in these immune complexes may be important for the design of mAb used for the immunotherapy of infectious diseases (Motley et al. 2020) or of various cancers (Waldman et al. 2020), where improved mAb binding affinity is desired under fever conditions.

Mining of antigen–antibody immune complexes

We identified 77 Protein Data Bank (PDB) crystal structures with epitopes from a variety of viruses (12 species), bacteria (8 species), protozoa (4 species) and cancer/auto-immune diseases (8 antigens), totaling 1890 contact residues. These entries were selected according to the following inclusion criteria:

1. The pathogen or the cancer elicits fever in humans, and clinical data for the fever values is available.
2. The antigen is soluble or surface-exposed, such that SPR or ELISA assays with pathogens are feasible.
3. All relevant antibodies are monoclonal, such that binding sites are known and temperature-dependent affinity constants could be determined in the literature.
4. Both the mAb and Ag proteins are commercially available, to facilitate testing.

Of these, only 33 PDBs with full kinetic information of immune complex formation are available (highlighted in Supplementary Table 1), in order to construct correlation matrices between fever maxima in patients and kinetic parameters. We have used the clinically recorded fever maxima for these conditions as a means to classify pathogens

Table 1 Frequency of the amino acids at the interfaces considered in this study. In brackets, the calculated percentages out of total amino acids in the interface. Highlighted in gray are the three most important contributions to interface formation within each category that are enriched when compared to the control dataset in Supplementary Table 2

	Viral epitopes	Paratopes against viruses	Bacterial epitopes	Paratopes against bacteria	Protoz. epitopes	Paratopes against protozoans	Cancer epitopes	Paratopes against cancers
Total number	215 AA	211 AA	228 AA	294 AA	209 AA	313 AA	187AA	233 AA
Asp	11 (5%)	27 (12.8%)	27 (11.8%)	26 (8.8%)	22 (10.5%)	24 (7.6%)	12 (6.4%)	18 (7.7%)
Glu	11 (5%)	10 (4.7%)	19 (8.3%)	4 (1.3%)	20 (9.5%)	9 (2.8%)	15 (8%)	10 (4.3%)
Lys	28 (13%)	3 (0.9%)	32 (14%)	4 (1.3%)	28 (13.4%)	16 (5.1%)	16 (8.5%)	4 (1.7%)
Arg	9 (4.1%)	16 (7.6%)	9 (4%)	14 (4.8%)	13 (6.2%)	18 (5.7%)	12 (6.4%)	9 (3.8%)
Ser	12 (5.6%)	31 (14.7%)	12 (5.3%)	36 (12.2%)	7 (3.3%)	40 (12.7%)	22 (11.7%)	34 (14.6%)
Thr	28 (13%)	15 (7.1%)	11 (4.8 %)	20 (6.8%)	8 (3.8%)	29 (9.2%)	6 (3.2%)	19 (8.1%)
Asn	13 (6%)	17 (8%)	26 (11.4 %)	26 (8.8%)	27 (12.9%)	15 (4.8%)	7 (3.7%)	19 (8.1%)
Gln	7 (3.3%)	0	12 (5.3%)	5 (1.7%)	9 (4.3%)	3 (0.9%)	9 (4.8%)	3 (1.2%)
Cys	4 (1.8%)	0	0	0	5 (2.4%)	0	4 (2.1%)	-
Gly	21 (9.7%)	11 (5.2%)	16 (7%)	9 (3%)	6 (2.8%)	8 (2.6%)	1 (0.5%)	6 (2.5%)
His	5 (2.3%)	1 (0.5%)	3 (1.3%)	16 (5.4%)	3 (1%)	16 (5.1%)	7 (3.7%)	8 (3.4%)
Trp	2 (0.9%)	10 (4.7%)	6 (2.6%)	20 (6.8%)	2 (0.7%)	23 (7.4%)	3 (1.6%)	13 (5.5%)
Tyr	14 (6.5 %)	40 (19%)	15 (6.5%)	76 (25.8%)	12 (5.7%)	58 (18.5%)	8 (4.3%)	56 (24%)
Val	5 (2.3%)	6 (2.8%)	11 (4.8 %)	4 (1.3%)	6 (2.8%)	10 (3.2%)	12 (6.4%)	6 (2.5%)
Ile	9 (4.1%)	10 (4.7%)	0	2 (0.7%)	5 (2.4%)	3 (0.9%)	12 (6.4%)	9 (3.8%)
Leu	7 (3.3%)	4 (1.9%)	10 (4.4%)	6 (2%)	6 (2.8%)	22 (7%)	5 (2.6%)	5 (2.1%)
Met	3 (1.4%)	1 (0.5%)	0	2 (0.7%)	3 (1%)	0	3 (1.6%)	1 (0.4%)
Phe	9 (4.1%)	3 (1.4%)	7 (3%)	9 (3%)	6 (2.8%)	8 (2.6%)	1 (0.5%)	6 (2.5%)
Ala	12 (5.6%)	3 (1.4%)	5 (2.1)	9 (3%)	12 (5.7%)	7 (2.2%)	12 (6.4%)	5 (2.1%)
Pro	5 (2.3%)	3 (1.4%)	7 (3%)	6 (2%)	9 (4.3%)	4 (1.2%)	20 (10.6%)	2 (0.8%)

and relevant cancers according to one measure of the febrile response. These maxima may also correlate with the severity of illness, as demonstrated in e.g. experimental shigellosis and dengue virus infection (Mackowiak et al. 1994, 1992). Furthermore, using these fever values may serve to customize whole-body heating therapies against different infections, procedures that are known to aid in pathogen clearance (Jiang et al. 1999). A list of the 77 PDB here considered, along with clinical markers (presence of fever, presence of periodic fever, maximal fever values, disease(s) involved and identity of amino acids under investigation) is shown in Supplementary Table 1.

For comparison, we have used as a control set 31 PDB of immune complexes formed between paratopes and epitopes of bacterial and viral targets that do not induce significant fever (> 38 °C) in humans (Supplementary Table 2). We have also added to this control group immune complexes having a wide range of epitopes such as the Von Willebrand, epidermal or vascular endothelial growth factors, CD3 and CD154, IL-10, angiogenin.

Manual curation of structures and thermodynamic data

We further restricted the sequence and structural analyses to residues situated within a 4Å distance between the paratopes and epitopes. The choice for this cutoff was based on an evolutionary model for the covariance of protein interfaces during evolution (Ovchinnikov et al. 2014). We acknowledge that the issue of choosing the appropriate cutoff to describe protein–protein interfaces is still debated (Kastritis and Bonvin 2012).

The identity of the amino acids was obtained from literature, with changes indicated in text. We further inspected the structures and measured the distances between residues using PyMol (Schrödinger, Inc.), in order to remove erroneous contacts, as indicated in Supplementary Table 1. We also used the primary literature source to obtain kinetic parameters of immune complex formation, whenever available. Of note, none of the quoted binding affinity constants were obtained at the relevant physiological or fever temperatures,

Table 2 Percentages of the interface amino acids with different structural and physico-chemical properties. Highlighted in gray are the two most important contributions to interface formation within each category that are enriched over the control dataset in Supplementary Table 3. The non-polar proline is presented separately

Property	Viral epitopes	Paratopes against viruses	Bacterial epitopes	Paratopes against bacteria	Protozoan epitopes	Paratopes against protozoans	Cancer epitopes	Paratopes against cancers
Hydrophobic	23.1%	14.1%	17.3%	12.7%	21.8%	17.1%	34.5%	14.2%
Positive charge	17.1%	8.5%	18%	6.1%	19.6%	10.8%	14.9%	5.5%
Negative charge	10%	17.5%	20.1%	10.1%	20%	10.4%	14.4%	12%
Polar	29.7%	29.8%	26.8%	29.5%	26.7%	27.6%	25.5%	32%
Small size	38%	31.2%	24%	14.1%	20.8%	29.9%	30.3%	29.8%
Extended length	26.8%	13.7%	31.6%	9.1%	34.4%	14.5%	29.3%	11.4%
Proline	2.3%	1.4%	3%	2%	4.3%	1.2%	10.6%	0.8%

but at 25 ℃ or at room temperatures. While these values enable comparisons between data, it is likely that they underestimate the values obtainable at higher, physiologically relevant temperatures (Schreiber et al. 2009).

Correlation matrices

Kinetic data of mAb binding to pathogens or cancer epitopes were obtained from literature (Supplementary Table 1). Correlation matrices for this data against the fever temperatures they provoke in patients were constructed using Prism 9.3.0. Spearman correlation coefficients were calculated assuming a two-tailed, non-Gaussian distribution, alpha = 0.05.

Statistical analyses

Comparisons between sets of epitope or paratope residues belonging to the main set (77 PDB) or the control set (31 PDB) were performed using the Mann–Whitney test in OriginPro 2021.

Identification and categorization of residue-residue interactions

Antigen–antibody binding interfaces are not homogenous. Certain interface residues contribute the most to the binding (hotspots), whereas the rest may have a secondary role (Kastritis and Bonvin 2012). We have identified the residues in the paratopes and epitopes of those immune complexes present in fever-inducing pathogens and relevant cancers. An overview of the distribution frequency of all residues in the interfaces of these immune complexes is indicated in Table 1.

Based on the frequency counts, Table 1 indicates that hotspots include tyrosine as the most abundant amino acids in all of the paratopes investigated, including in the control set, with the exception of viral paratopes therein (Supplementary Table 2). This enhanced presence of tyrosine at the protein interfaces is a general phenomenon (Akbar et al. 2021). Such feature may be due to its amphipathic and large side chain, capable of multiple interactions (cation-π or H-bonding), while its planar rigidity allows for both increased specificity and smaller entropy penalty when bound, overall increasing the thermal stability of the binding complex (Koide and Sidhu 2009). For the epitopes, lysine is the highest represented amino acid in pathogens, whereas proline is preponderant in cancers. In contrast, in the control set, lysine is not the prevalent residue in the bacterial and viral epitopes, nor in the heterogeneous group of non-tumor antigens. Pertinently, cation-π bonds that form between positively charged residues such as lysine and aromatic residues, in particular with tyrosine, have been suggested (Wang et al. 2018) to be of importance in the thermal stabilization of protein complexes (Chakravarty and Varadarajan 2002). While tyrosine has been repeatedly found to be the predominant amino acid in paratopes, the next residue that is most enriched in these structures is aspartic acid (Wang et al. 2018), unlike in our target dataset where it is replaced by serine. Correspondingly, in the control data set, serine is also the second most frequent residue in the paratopes against bacterial and viral epitopes; however, in the paratopes against non-cancer targets, that position is occupied by asparagine (Supplementary Table 2).

In cancer epitopes, serine is the preponderant residue at these interfaces, while not being present in the same position in any of the categories from the control set (Supplementary Table 2). Importantly, this polar-uncharged

residue has been found to most destabilize proteins against temperature changes (Ponnuswamy et al. 1982), an observation that may be relevant for establishing new epitopes in proteins relevant for cancer and auto-immune diseases. It is notable to encounter proline in cancer epitopes (Table 1), as this residue is a known disruptor of protein secondary structures. Proline is, moreover, a very common residue in proteins adapted to high temperatures, where it is known to prevent unfolding, independent of the residues it contacts (Saelensminde et al. 2009); in contrast, the control dataset uses glutamine as the most represented residue at the epitopes of non-tumor antigens.

It is important also to consider the overall thermodynamic and structural properties of all residues encountered in the interfaces, as shown in Table 2, and to compare these features against the control set (Supplementary Table 3).

A common feature observed in these data is that a combination of structural factors (size of residues) in conjunction with polarity with respect to water accounts in general for more than the 50% of the necessary binding energy (Table 2). As such, polar residues are important in all paratopes of the complexes here considered. Compared to hydrophobic interactions, polar interactions are more directional in character as they are either charged at physiological pH or can participate in hydrogen bonding (Bolon and Mayo 2001). On average, for protein–protein complexes, approximately 70% of the interfacial residues are estimated to be hydrophilic (Kastritis and Bonvin 2012). It has been proposed that for the formation of immune complexes to occur, these have to be first stabilized by long-range electrostatic interactions that can extend as far as 10 Å (Zhou and Pang 2018). Within these interactions, hydrogen bonding is predominant and imposes the initial partner specificity (Schreiber 2020). It has also been suggested that electrostatic interactions are mainly used to filter out unbound proteins rather than increasing the affinity for target proteins (André et al. 2004). Hydrogen bonding is exothermic and thus more stable at lower temperatures (Reverberi and Reverberi 2007), whereas the strength of the hydrophobic bonds tends to increase with increasing temperature (van Dijk et al. 2015). In the immune complexes raised against the viral epitopes, we have not observed significant differences in charged residues compared to the control set. In that category, however, a much higher proportion of hydrophobic residues is found in paratopes compared to control, whereas smaller residues dominate the epitopes, compared to longer extended amino acids in the control set. A similar situation is present in the immune complexes containing the bacterial epitopes, in that paratope hydrophobic residues are dominant for binding, whereas no significant differences in terms of charge or residue size are found against the control set. For the protozoan set that lacks a control, we find a larger proportion of hydrophobic residues (by a factor of 2 difference) compared to protozoal epitopes; we also found one factor of difference between charged amino acid epitopes and charged paratope residues. The pattern of high content of hydrophobic paratope residues in immune complexes pertinent to fever is also found in the cancer data set, with even more striking distinction compared to the control set (a factor of 4 difference). Similarly to the dataset containing bacterial epitopes, in terms of charge and size, no other important variation was found between the two datasets in this category. It is important here to note that while specificity of interactions relies on salt bridge formation and hydrogen bonding that can contribute to protein complex stability, this effect is counter-balanced by unfavorable desolvation that might destabilize the immune complex (Wong et al. 2013). In contrast, hydrophobic surfaces bind much faster than hydrophilic ones, since trapped water creates a barrier to rapid assembly (Kastritis and Bonvin 2012; Reddy et al. 2010).

As noted, other than polarity, the size of the residues appears to be strongly correlated to the formation of the immune complexes. All paratopes, along with viral and cancer epitopes, make use of small residues, especially Ser and Thr (each with volumes under 120 cubic Å) (Pommié et al. 2004). On the other hand, bacterial and protozoan epitopes use long residues, in particular Lys and Arg, each with volumes under 175 cubic Å, for the formation of interfaces. Longer amino acids are favorable for thermal stabilization of interfaces, as they can establish more van der Waals interactions, and, markedly, bacterial and protozoan infections cause the highest fever values in patients (Supplementary Table 1).

At higher temperatures, the entropic factor will take preponderance over the enthalpy as the main driver of binding (Wang et al. 2018), as observed with other immune complexes. We observed a much lower average percentage of hydrophobic residues in the binding interfaces in our data, ranging between 12 to 23% of all interface amino acids for most systems, compared to data obtained from larger sample sizes of around 50% (Nguyen et al. 2017). Evolutionarily, proteins from species adapted to higher temperatures contain cores that are more hydrophobic, while the surface is on average more hydrophilic and charged (Saelensminde et al. 2007). It is interesting to note in this context that the largest percentage of hydrophobic residues (~ 34%) was observed in epitopes relevant for cancers, suggesting that hidden epitopes might be accessible through temperature changes.

We further confirmed these results by comparing the datasets of residues in the interfaces of the immune partners that are involved in the fever response against those that are not, in order to quantify the extent of amino acid enrichment in each category. First, we established that the distributions of amino acids in the epitopes and paratopes from the main dataset

are statistically significantly different than the epitopes and paratopes, respectively, from the control dataset (Supplementary Tables 4 and 5). We then calculated and plotted the differences between the percentages of representation of the residues of the epitopes and the paratopes within each of the three categories (viral, bacterial and tumor versus non-tumor complexes), as shown in Supplementary Fig. 1. For the viral epitopes, using the fever dataset against the control dataset, we confirmed the same enrichment in lysine, threonine and glycine, whereas, equally, for the paratopes against viral targets, tyrosine is over-represented. Similarly, for the epitopes from the bacterial main dataset, compared with the controls, we confirmed that lysine, aspartic acid and asparagine are the most enriched, whereas isoleucine and alanine are most prevalent in the control set. As with most antibodies, tyrosine is the most enriched residue in the paratopes raised against bacteria that are also involved in the fever response, whereas glutamic acid is its counterpart in the paratopes raised against bacteria which do not induce a significant fever response. Interestingly, in the case of the epitopes from the cancer dataset, proline, and not serine, is dominant in the main dataset, insomuch as glycine and glutamine dominate the control dataset. Conversely, for the paratopes raised against cancer targets, histidine is the most represented residue, whereas tyrosine is not. It should be noted, however, that histidine might be over-represented due to its rare occurrence in the antibody datasets in general (Chen et al. 2009). For those paratopes targeting non-cancer epitopes from the control dataset, however, after tyrosine, asparagine and glycine are dominant compared to the amino acids from the main cancer dataset.

Kinetic parameters of immune complexes formation and correlations with febrile temperatures in patients

The effect of temperature is characteristic for each antigen–antibody complex and will differentially influence the binding and dissociation (undocking) rate constants (Kourentzi et al. 2008). As such, increases in temperature will cause an increase in both association and dissociation rates of the antibodies (Johnstone et al. 1990). Of these, the off-rate (K_{off}) data are expected to be more accurate as to their strict dependence on temperature. While association rates (K_{on}) are also influenced by temperature, the concentrations of antibodies and antigens have an important role in their determination (Schwesinger et al. 2000). Furthermore, most of the determined kinetic constants compiled from literature were obtained using Surface Plasmon Resonance, where analyte diffusion can play an important role in obtaining correct K_{on}, but is not relevant for K_{off} values. K_{on} and K_d depend on a thermal control of diffusion, with higher temperatures increasing protein encounters until docking has occurred. We identified the dissociation constants (K_d) along with the subset containing also the K_{on} and K_{off} rates (33 entries out of the 64). Avidity effects, if present, are mentioned in the Supplementary Table 1. Figure 1 below presents an overview of the binding constants available with respect to the fever recorded for the clinical condition that each PDB belongs to.

K_d values for the cancer and bacterial data cluster relatively at the lower spectrum or at the higher spectrum of fever temperatures (38 ℃ and 42 ℃, respectively). We also note that most of the values are restricted within the 38 to 40 °C range, which may relate to the upper thermal tolerance limit before hyperthermia occurs, leading to multiple organ failure (Becker and Stewart 2011). Importantly, infections caused by pathogens tend to produce short but intense bouts of fever, whereas those cancers that do have a fever component are of low intensity and persistent i.e. weeks to months (Foggo and Cavenagh 2015). The K_d values for the immune complexes formed with viral epitopes are centered around 40 ℃, which may also relate to the viral life cycle between hosts having an optimal temperature for replication in the mammalian host. For instance, infectivity of influenza virus is more effective at a temperature around 37 °C or lower, where lipid ordering leads to increased viral stability. Such stability is required for viral airborne transmission, whereas viral

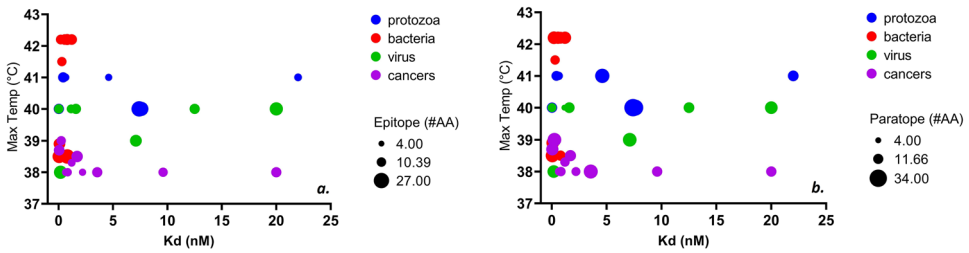

Fig. 1 Summary of K_d constants obtained from binding studies for pathogens and cancer epitopes in complexes with mAb, plotted against febrile temperatures for each pathology and against the length of epitopes (**a**) and paratopes (**b**). Maximal fever values are detailed in Supplementary Table 1 and are not the temperatures that the binding assays were performed at. The symbol size is proportional to the number of amino acid residues on the Ag-Ab contact interface that are involved in complex formation

clumping on epithelial cell surfaces was observed at 41 °C that may adversely affect infectivity (Polozov et al. 2008).

It is important to consider whether a relatively small temperature increase from core body temperature by only 1 to 4 °C can affect the activity of the proteins involved in the immune response. In general, simulating or experimentally assessing the folding equilibrium or the binding affinity of large proteins in the crowded environment and under different temperature conditions has been challenging. Unfolding and refolding at 39 °C of the phosphoglycerate kinase in human osteosarcoma and cervical carcinoma cells, using time-resolved fluorescence imaging with fast temperature jump–induced kinetics, showed that proteins were more stable, that the thermal denaturation was more gradual and the folding kinetics was slower in the test cells than in vitro conditions (Ebbinghaus et al. 2010). The main difference is a slightly steeper temperature dependence of the folding rate in some cells that can be rationalized in terms of temperature-dependent crowding and local viscosity effects (Guo et al. 2012). A study involving 17 antibiotics and 432 pathogenic Gram positive and negative bacterial strains has revealed a progressive increase in the anti-bacterial activity with temperature, from the standard 35 °C onto 37 °C, 38.5 °C, 40 °C and peaking at 41.5 °C (Mackowiak et al. 1982). It is significant to note here that these febrile temperatures alone did not alter significantly the bacterial growth. Rather, the loss of resistance to antibiotics was due to inactivation of protective enzymes such as penicillinase (Asheshov 1966). When cell-mediated immunity is assessed in vitro at 39 °C, compared to physiological temperatures, increased antigen stimulation was measured against sera from *Mycobacterium tuberculosis* infected patients (Aabye et al. 2011). In vitro studies have shown that mAb significantly increase their binding affinity against dengue epitopes and protozoal *Plasmodium vivax* epitopes at 40 °C and that, conversely, a higher temperature (42 °C) may adversely affect antibody binding, possibly due to unfolding (Stan et al. 2019). For dengue infection, 37 °C represents the relevant temperature point for viral entry into human hosts, whereas at 40 °C, DENV1 shows remarkable expansion in its outermost shell, especially in its envelope E-protein components (Lim et al. 2017). At this higher temperature, mAb that thus target fully or partially exposed cryptic epitopes are essentially "temperature-dependent antibodies" (Lok et al. 2008). Febrile temperature-dependent viral "breathing" that allowed exposure of new epitopes and enhanced capture by neutralizing mAb has been measured at 40 °C for hepatitis C virus, compared to measurements at the physiological temperature (Sabo et al. 2012). Furthermore, temperature-dependent conformational flexibility of flaviviruses that impacts sensitivity to antibody-mediated neutralization, through changes in epitope accessibility, has also been observed for West Nile Virus infections at 40 °C, compared to 37 °C (Dowd et al. 2011).

Conclusions

In critically ill patients, any advantage in the fight against infections or cancers may be essential. Fever is an evolutionarily conserved and beneficial trait that modulates, among others, the immune responses to infections. Febrile temperatures lead to Th2 skewing by CD4 T lymphocytes, and to subsequent counter signaling by dendritic cells via overexpression of IL-12 (Umar et al. 2020). Activation of CD8 + T cells and enhanced antitumor response has also been measured under fever conditions (O'Sullivan et al. 2021). Furthermore, fever temperatures increase T lymphocyte trafficking to the draining lymph nodes and improve bacterial clearance (Lin et al. 2019). Despite generating an exceedingly large number of interactions, the paratope-epitope interactions are based on a finite vocabulary of interaction motifs (Wang et al. 2018) which appear to be universal (Akbar et al. 2021). In this work, we propose that fever might also be beneficial for the organisms by increasing mAb binding affinity. We observed a marked enrichment in hydrophobic residues in all the paratopes here investigated, but not in the control set. We found that epitopes are differentially enriched, such that lysine are dominant in the pathogenic epitopes, possibly in order to form cation-π bonds to tyrosine, the most dominant residue in all paratopes; we do not find these occurrences in the control data set. Finally, in the cancer epitopes, where the fever values are generally the lowest in our data, the presence of serine and proline may suggest disruptions in the protein structure that could lead to new epitopes being formed. In this case, fever may act adversely to expose epitopes that could contribute to cancer pathology.

This characteristic may be manifested by a mechanism of action whereby antigen recognition relies on an "induced fit" model (Wang et al. 2018; Lancet and Pecht 1976). This plasticity feature of the paratopes would allow for a convergence of specificities to a subset of antigenic determinants (Akbar et al. 2021), without the metabolic cost of generating a unique mAb for any possible antigen (Torres et al. 2007). As antibodies do not rely on ATP use for antigen binding, the conformational space an antibody explores may also be directly modulated by temperature, with higher temperatures enabling the surmount of kinetic traps in the energetic landscape. We anticipate that ongoing experiments aimed at detecting the effect of fever temperatures on these immune complexes will establish the potential benefit to improving binding affinities, with immediate application to monoclonal Ab engineering.

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Declarations

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