1. Bayry, J.; Lacroix-Desmazes, S.; Pashov, A.; Stahl, D.; Hoebeke, J.; Kazatchkine, M. D.; Kaveri, S. V., Autoantibodies to factor VIII with catalytic activity. Autoimmun Rev 2003, 2, (1), 30-35.

Hemophilia A is an X-linked, recessive, bleeding disorder caused by defective or deficient factor VIII (FVIII) molecules. Infusion of purified FVIII to patients with severe hemophilia A results in approximately 25% of the cases, in the emergence of anti-FVIII antibodies (inhibitors) that are known to neutralize the pro-coagulant activity of FVIII by steric hindrance. We recently reported on the proteolysis of FVIII by allo-antibodies in the plasma of high responder patients with severe hemophilia A, demonstrating a new mechanism by which FVIII inhibitors may prevent the pro-coagulant function of FVIII. Hemophilia is the first model where a direct link between the hydrolysis of the target molecule and the occurrence of the clinical manifestations may be established. It also represents the first example in humans, of the induction of catalytic antibodies following the exogenous administration of an antigen. The characterization of FVIII inhibitors as site-specific proteases may provide new approaches to the treatment of inhibitors.

2. Dimitrov, J. D., C. Planchais, J. Kang, A. Pashov, T. L. Vassilev, S. V. Kaveri, S. Lacroix-Desmazes. Heterogeneous antigen recognition behavior of induced polyspecific antibodies. Biochemical and Biophysical Research Communications 398 (2010) (2),266-271.

Polyspecific antibodies represent a significant fraction of the antibody repertoires in healthy animals and humans. Interestingly, certain antibodies only acquire a polyspecific antigen-binding behavior after exposure to protein-modifying conditions, such as those found at inflammation sites, or used in small- and large-scale immunoglobulin purification. This phenomenon is referred to as "criptic polyspecificity". In the present study, we compare the potential of different chemical agents to induce IgG polyspecificity. Depending on the treatment used, quantitative and qualitative differences in the recognition of individual antigens from a standard panel were observed. Antibodies with cryptic polyspecificity utilized common mechanisms for the recognition of structurally unrelated antigens when exposed to a particular inductor of polyspecificity. Our study contributes to the understanding of the mechanisms underlying the cryptic polyspecificity.

3. Djoumerska-Alexieva, I.; Roumenina, L.; Pashov, A.; Dimitrov, J.; Hadzhieva, M.; Lindig, S.; Voynova, E.; Dimitrova, P.; Ivanovska, N.; Bockmeyer, C.; Stefanova, Z.; Fitting, C.; Blass, M.; Claus, R.; Von Gunten, S.; Kaveri, S.; Cavaillon, J. M.; Bauer, M.; Vassilev, T., Intravenous Immunoglobulin with Enhanced Polyspecificity Improves Survival in Experimental Sepsis and Aseptic Systemic Inflammatory Response Syndromes. Molecular medicine (Cambridge, Mass 2015.

Sepsis is a major cause for death worldwide. Numerous interventional trials with agents neutralizing single pro-inflammatory mediators have failed to improve survival in sepsis and aseptic systemic inflammatory response syndromes. This failure could well be explained by the widespread gene expression dysregulation known as "genomic storm" in these patients. A multifunctional polyspecific

therapeutic agent might be needed to thwart the effects of this "storm". Licensed pooled intravenous immunoglobulin preparations seemed to be a promising candidate but they have also failed in their present form to prevent sepsis-related death. We report here the protective effect of a single dose of intravenous immunoglobulin preparations with additionally enhanced polyspecificity in three models of sepsis and aseptic systemic inflammation. The modification of the pooled immunoglobulin G molecules by exposure to ferrous ions resulted in their newly acquired ability to bind some pro-inflammatory molecules, complement components and endogenous "danger" signals. The improved survival in endotoxemia was associated with serum levels of pro-inflammatory cytokines, diminished complement consumption and normalization of the coagulation time. We suggest that intravenous immunoglobulin preparations with additionally enhanced polyspecificity have a clinical potential in sepsis and related systemic inflammatory syndromes.

4. Ferdinandov, D., V. Kostov, M. Hadzhieva, V. Shivarov, P. Petrov, A. Bussarsky, A. D. Pashov. Reactivity Graph Yields Interpretable IgM Repertoire Signatures as Potential Tumor Biomarkers. International Journal of Molecular Sciences 24 (2023) (3),2597.

Combining adaptive and innate immunity induction modes, the repertoire of immunoglobulin M (IgM) can reflect changes in the internal environment including malignancies. Previously, it was shown that a mimotope library reflecting the public IgM repertoire of healthy donors (IgM IgOme) can be mined for efficient probes of tumor biomarker antibody reactivities. To better explore the interpretability of this approach for IgM, solid tumor-related profiles of IgM reactivities to linear epitopes of actual tumor antigens and viral epitopes were studied. The probes were designed as oriented planar microarrays of 4526 peptide sequences (as overlapping 15-mers) derived from 24 tumor-associated antigens and 209 cancer-related B cell epitopes from 30 viral antigens. The IgM reactivity in sera from 21 patients with glioblastoma multiforme, brain metastases of other tumors, and non-tumor-bearing neurosurgery patients was thus probed in a proof-of-principle study. A graph representation of the binding data was developed, which mapped the cross-reactivity of the mixture of IgM (poly)specificities, delineating different antibody footprints in the features of the graph—neighborhoods and cliques. The reactivity graph mapped the major features of the IgM repertoire such as the magnitude of the reactivity (titer) and major cross-reactivities, which correlated with blood group reactivity, non-self recognition, and even idiotypic specificities. A correlation between an aspect of this image of the IgM IgOme, namely, small cliques reflecting rare self-reactivities and the capacity of subsets of the epitopes to separate the diagnostic groups studied was found. In this way, the graph representation helped the feature selection in its filtering step and provided reduced feature sets, which, after recursive feature elimination, produced a classifier containing 51 peptide reactivities separating the three diagnostic groups with an unexpected efficiency. Thus, IgM IgOme approaches to repertoire studies is greatly augmented when self/viral antigens are used and the data are represented as a reactivity graph. This approach is most general, and if it is applicable to tumors in immunologically privileged sites, it can be applied to any solid tumors, for instance, breast or lung cancer.

5. Gorshkova, E. N.; Pashova, S.; Vasilenko, E. A.; Tchurina, T. S.; Razzorenova, E. A.; Starkina, O. V.; Dimitrova, P.; Pashov, A.; Vassilev, T. L., Induced Polyspecificity of Human Secretory Immunoglobulin A Antibodies: Is It Possible to Improve Their Ability to Bind Pathogens? Pharmacology 2022, 1-10.

INTRODUCTION: As has been shown previously, various protein-modifying agents can change the antigen-binding properties of immunoglobulins. However, induced polyspecificity of human secretory immunoglobulin A (sIgA) has not been previously characterized in detail. METHODS: In the present study, human secretory immunoglobulin A (IgA) was exposed to buffers with acidic pH, to free heme, or to pro-oxidative ferrous ions, and the antigen-binding behavior of the native and modified IgA to viral and bacterial antigens was compared using Western blotting and enzyme-linked immunosorbent assay. The ability of these agents to modulate the antigen-binding properties of human sIgA toward a wide range of pathogen peptides was investigated using an epitope microarray. RESULTS: We have shown that acidic pH, heme, and pro-oxidative ferrous ions influenced the binding of secretory IgA in opposite directions (either increasing or decreasing); however, the strongest effect was observed when using buffers with low pH. This fraction had the highest number of affected reactivities; most of them were increased and most of the new ones were toward common pathogens. CONCLUSIONS: Thus, it was shown that all investigated treatments can alter to some degree the antigen-binding of secretory IgA, but acidic pH has the most potentially beneficial effect by increasing binding to a largest number of common pathogens' antigens.

6. Hadzhieva, M., A. D. Pashov, S. Kaveri, S. Lacroix-Desmazes, H. Mouquet, J. D. Dimitrov. Impact of Antigen Density on the Binding Mechanism of IgG Antibodies. Scientific reports 7 (2017) (1),3767.

The density and distribution pattern of epitopes at the surface of pathogens have a profound impact on immune responses. Although multiple lines of evidence highlight the significance of antigen surface density for antibody binding, a quantitative description of its effect on recognition mechanisms is missing. Here, we analyzed binding kinetics and thermodynamics of six HIV-1 neutralizing antibodies as a function of the surface density of envelope glycoprotein gp120. Antibodies that recognize gp120 with low to moderate binding affinity displayed the most pronounced sensitivity to variation in antigen density, with qualitative and substantial quantitative changes in the energetics of the binding process as revealed by non-equilibrium and equilibrium thermodynamic analyses. In contrast, the recognition of gp120 by the antibodies with the highest affinity was considerably less influenced by variations in antigen density. These data suggest that a lower affinity of antibodies permits higher dynamics during the antigen recognition process, which may have considerable functional repercussions. These findings contribute to a better understanding of the mechanisms of antigen recognition by antibodies. They are also of importance for apprehending the impact of antigen topology on immune-defense functions of antibodies.

7. Hennings, L., C. Artaud, F. Jousheghany, B. Monzavi-Karbassi, A. Pashov, T. Kieber-Emmons. Carbohydrate Mimetic Peptides Augment Carbohydrate-Reactive Immune Responses in the Absence of Immune Pathology. Cancers 3 (2011) (4),4151-4169.

Among the most challenging of clinical targets for cancer immunotherapy are Tumor Associated Carbohydrate Antigens (TACAs). To augment immune responses to TACA we are developing carbohydrate mimetic peptides (CMPs) that are sufficiently potent to activate broad-spectrum anti-tumor reactivity. However, the activation of immune responses against terminal mono- and disaccharide constituents of TACA raises concerns regarding the balance between "tumor destruction" and "tissue damage", as mono- and disaccharides are also expressed on normal tissue. To support the development of CMPs for clinical trial testing, we demonstrate in preclinical safety assessment studies in mice that vaccination with CMPs can enhance responses to TACAs without mediating tissue damage to normal cells expressing TACA. BALB/c mice were immunized with CMPs that mimic TACAs reactive with Griffonia simplicifolia lectin 1 (GS-I), and tissue reactivity of serum antibodies were compared with the tissue staining profile of GS-I. Tissues from CMP immunized mice were analyzed using hematoxylin and eosin stain, and Luxol-fast blue staining for myelination. Western blots of membranes from murine mammary 4T1 cells, syngeneic with BALB/c mice, were also compared using GS-I, immunized serum antibodies, and naive serum antibodies. CMP immunization enhanced glycan reactivities with no evidence of pathological autoimmunity in any immunized mice demonstrating that tissue damage is not an inevitable consequence of TACA reactive responses.

8. Kieber-Emmons, T.; Monzavi-Karbassi, B.; Pashov, A.; Saha, S.; Murali, R.; Kohler, H., The promise of the anti-idiotype concept. Frontiers in oncology 2012, 2, 196.

A basic tenet of antibody-based immunity is their specificity to antigenic determinates from foreign pathogen products to abnormal cellular components such as in cancer. However, an antibody has the potential to bind to more than one determinate, be it an antigen or another antibody. These observations led to the idiotype network theory (INT) to explain immune regulation, which has wax and waned in enthusiasm over the years. A truer measure of the impact of the INT is in terms of the ideas that now form the mainstay of immunological research and whose roots are spawned from the promise of the anti-idiotype concept. Among the applications of the INT is understanding the structural implications of the antibody-mediated network that has the potential for innovation in terms of rational design of reagents with biological, chemical, and pharmaceutical applications that underlies concepts of reverse immunology which is highlighted herein.

9. Kieber-Emmons, T.; Saha, S.; Pashov, A.; Monzavi-Karbassi, B.; Murali, R., Carbohydrate-mimetic peptides for pan anti-tumor responses. Front Immunol 2014, 5, 308.

Molecular mimicry is fundamental to biology and transcends to many disciplines ranging from immune pathology to drug design. Structural characterization of molecular partners has provided insight into the origins and relative importance of complementarity in mimicry. Chemical complementarity is

easy to understand; amino acid sequence similarity between peptides, for example, can lead to cross-reactivity triggering similar reactivity from their cognate receptors. However, conformational complementarity is difficult to decipher. Molecular mimicry of carbohydrates by peptides is often considered one of those. Extensive studies of innate and adaptive immune responses suggests the existence of carbohydrate mimicry, but the structural basis for this mimicry yields confounding details; peptides mimicking carbohydrates in some cases fail to exhibit both chemical and conformational mimicry. Deconvolution of these two types of complementarity in mimicry and its relationship to biological function can nevertheless lead to new therapeutics. Here, we discuss our experience examining the immunological aspects and implications of carbohydrate-peptide mimicry. Emphasis is placed on the rationale, the lessons learned from the methodologies to identify mimics, a perspective on the limitations of structural analysis, the biological consequences of mimicking tumor-associated carbohydrate antigens, and the notion of reverse engineering to develop carbohydrate-mimetic peptides in vaccine design strategies to induce responses to glycan antigens expressed on cancer cells.

10. Kohler, H., A. Pashov, T. Kieber-Emmons. The Promise of Anti-idiotype Revisited. Frontiers in Immunology 10 (2019) (808).

The promise of idiotype-based therapeutics has been disappointing forcing a new look at the concept and its potential to generate an effective approach for immunotherapy. Here, the idiotype network theory is revisited with regard to the development of efficacious anti-idiotype vaccines. The experience of polyclonal anti-Idiotype reagents in animal models as well as an understanding of the immune response in humans lends to the proposition that polyclonal anti-Idiotype vaccines will be more effective compared to monoclonal-based anti-Idiotype vaccines. This novel strategy can be adapted in Biotech-standard production of therapeutic antibodies

11. Lecerf, M.; Scheel, T.; Pashov, A. D.; Jarossay, A.; Ohayon, D.; Planchais, C.; Mesnage, S.; Berek, C.; Kaveri, S. V.; Lacroix-Desmazes, S.; Dimitrov, J. D., Prevalence and Gene Characteristics of Antibodies with Cofactor-induced HIV-1 Specificity. J Biol Chem 2015.

The healthy immune repertoire contains a fraction of antibodies that bind to various biologically relevant cofactors, including heme. Interaction of heme with some antibodies results in induction of new antigen-binding specificities and acquisition of binding polyreactivity. In vivo, extracellular heme is released as a result of hemolysis or tissue damage, hence the post-translational acquisition of novel antigen specificities might play an important role in the diversification of the immunoglobulin repertoire and host defense. Here, we demonstrate that seronegative immune repertoires contain antibodies that gain reactivity to HIV-1 gp120 upon exposure to heme. Furthermore, a panel of human recombinant antibodies was cloned from different B-cell subpopulations and the prevalence of antibodies with cofactor-induced specificity for gp120 determined. Our data reveal that upon exposure to heme, approximately 24% of antibodies acquired binding specificity for divergent strains of HIV-1 gp120. Sequence analyses reveal that heme-sensitive antibodies do not differ in their repertoire of variable

region genes and in most of the molecular features of their antigen-binding sites, from antibodies that do not change their antigen-binding specificity. However, antibodies with cofactor-induced gp120 specificity possess significantly lower numbers of somatic mutations in their variable-region genes. This study contributes to the understanding of the significance of cofactor-binding antibodies in immunoglobulin repertoires and of the influence that the tissue microenvironment might have in shaping adaptive immune responses.

12. Monzavi-Karbassi, B.; Hennings, L. J.; Artaud, C.; Liu, T.; Jousheghany, F.; Pashov, A.; Murali, R.; Hutchins, L. F.; Kieber-Emmons, T., Preclinical studies of carbohydrate mimetic peptide vaccines for breast cancer and melanoma. Vaccine 2007, 25, (16), 3022-3031.

Limited immune responses to tumor-associated carbohydrate antigens (TACA) are due in part to their being self-antigens. Immunization with xenoantigens of TACA provides an approach to break tolerance and augment responses to TACA. Carbohydrate mimetic peptides (CMPs) as xenoantigens can induce serum antibodies that target shared carbohydrate residues on differing carbohydrate structures. In preclinical studies, we observe that CMP immunization in mice induce immune responses that are effective in inhibiting the in vitro and in vivo growth of breast cancer and melanoma tumor cells expressing self-target antigens. CMPs of TACA can be further defined that induce IgM antibodies with broadened responses to both breast and melanoma cells. Consequently, CMPs are effective at generating a multifaceted carbohydrate-reactive immune response that should be clinically evaluated for their ability to amplify carbohydrate immune responses against circulating or disseminated tumor cells.

13. Monzavi-Karbassi, B.; Pashov, A.; Jousheghany, F.; Artaud, C.; Kieber-Emmons, T., Evaluating strategies to enhance the anti-tumor immune response to a carbohydrate mimetic peptide vaccine. Int J Mol Med 2006, 17, (6), 1045-52.

Carbohydrate mimetic peptides of tumor associated carbohydrate antigens (TACA) are T-cell-dependent antigens and, therefore, immunization with these surrogates is predicted to overcome the low immunogenicity of carbohydrate antigens. Consistent with this hypothesis, we show that among the potential immune cells involved, peptide immunization led to an increase in T-cell populations. While peptide mimetics may also function as TLR binding ligands, we did not observe evidence of involvement of NK cells. Examining tumor challenged animals, we observed that peptide immunization and not tumor cells rendered IL-12 responsiveness to T-cells, as T-cells from peptide-immunized mice produced IFN-gamma upon stimulation with IL-12. Cyclophosphamide administration enhanced the anti-tumor efficacy of the vaccine, which was achieved by enhancing T-cell responses with no effect on NK cell population. Prophylactic immunization of mice with a DNA construct encoding carbohydrate mimetic peptides indicated a specific role for the mimotope vaccine in anti-tumor immune responses. These data suggest a role for both CD4(+) and CD8(+) T-cells induced by mimotopes of TACA in protective immunity against tumor cells.

14. Pashov, A.; Monzavi-Karbassi, B.; Kieber-Emmons, T., Glycan mediated immune responses to tumor cells. Hum Vaccin 2011, 7, 156-65.

Preclinical animal studies convincingly demonstrate that tumor immunity to self-antigens can be actively induced and can translate into effective anti-tumor responses. Among the most challenging of clinical targets for cancer immunotherapy is Tumor Associated Carbohydrate Antigens (TACA). The molecular characterization of TACA suggest that these glycans are both altered and self-antigens. A new appreciation of the interaction of glycans with immune effector cells that will benefit immunotherapy strategies is emerging as more information on the nature of molecular interactions of glycan recognition molecules is obtained. Carbohydrate recognition affects more or less every aspect of the innate and adaptive immune response and their role in immunotherapy of cancer should be considered beyond the existing paradigm of traditional TACA based-vaccines.

15. Pashov, A.; Monzavi-Karbassi, B.; Raghava, G. P.; Kieber-Emmons, T., Bridging innate and adaptive antitumor immunity targeting glycans. J Biomed Biotechnol 2010, 2010, 354068.

Effective immunotherapy for cancer depends on cellular responses to tumor antigens. The role of major histocompatibility complex (MHC) in T-cell recognition and T-cell receptor repertoire selection has become a central tenet in immunology. Structurally, this does not contradict earlier findings that T-cells can differentiate between small hapten structures like simple glycans. Understanding T-cell recognition of antigens as defined genetically by MHC and combinatorially by T cell receptors led to the "altered self" hypothesis. This notion reflects a more fundamental principle underlying immune surveillance and integrating evolutionarily and mechanistically diverse elements of the immune system. Danger associated molecular patterns, including those generated by glycan remodeling, represent an instance of altered self. A prominent example is the modification of the tumor-associated antigen MUC1. Similar examples emphasize glycan reactivity patterns of antigen receptors as a phenomenon bridging innate and adaptive but also humoral and cellular immunity and providing templates for immunotherapies.

16. Pashov, A., R. Murali. Should All Memory B Cells Recruited to the Germinal Center Be Antigen Specific? Monoclonal antibodies in immunodiagnosis and immunotherapy 40 (2021) (2),50-51.

Viant et al. (2020) report that fate-mapped memory B cells generated during responses to NP-OVA or HIV-1 TM4-Core show surprisingly little enrichment for antigen-specific clones, implying that the memory compartment is dominated by extremely low-affinity binders detectable only when assay avidity is artificially increased. While provocative, this conclusion rests heavily on assay choice and sensitivity rather than on an unambiguous biological readout of specificity. Their S1pr2-ERT2cre fate mapping is interpreted as selectively labeling GC-derived progeny, yet the unexpectedly low “specificity” among labeled memory cells raises the possibility of technical under-detection, thresholding artifacts, or incomplete alignment between the labeling window and true GC commitment. The affinity comparisons—234 recombinant Fabs measured primarily by biolayer interferometry—further risk undercalling weak interactions because BLI is substantially less sensitive than surface plasmon

resonance, and the study emphasizes *KD* while omitting dissociation kinetics koff, a parameter often more predictive of functional engagement. Moreover, the absence of correlation between mutation load and measured binding could reflect measurement floor effects rather than genuine decoupling of somatic hypermutation from selection. Finally, the key mechanistic question is left open: are these "low-affinity" memory antibodies narrowly monospecific or broadly polyspecific/cross-reactive, which would radically change the interpretation from defective selection to adaptive breadth.

17. Pashov, A., R. Murali, I. Makhoul, B. Karbassi, T. Kieber-Emmons. Harnessing Antibody Polyspecificity for Cancer Immunotherapy. Monoclonal antibodies in immunodiagnosis and immunotherapy 41 (2022) (5),290-300.

Targeting the diverse glycan repertoire expressed on tumor cells is considered a viable therapeutic strategy to deal with tumor cell heterogeneity. Inherently polyspecific, natural, glycan-reactive antibodies are purported to be protective in thwarting infections and in cancer immunotherapy. Tumor-associated carbohydrate antigens (TACAs) are related to pathogen glycans, to which nascent or natural antibodies exist and IgM responses are elicited. To capture the polyspecific nature of anticarbohydrate responses, we have focused on the rational design of carbohydrate mimetic peptides (CMPs) cross-reactive with TACA reactive antibodies. In particular, we have focused on the development of CMPs that display reactivity to GD2 and Lewis Y (LeY) reactive monoclonal antibodies. They would serve as templates for pan-immunogens inducing biosimilar polyreactive antibodies. In the design, we relied on structural analyses of CMP's enhanced binding to the templates using molecular modeling. Glycan reactivity patterns of affinity CMP-purified human antibodies further refined specificity profiles in comparison with the immune response to the CMP in clinical trials. In this study, we further define the molecular characteristics for this mimicry by considering the polyspecificity of LeY and GD2 reactive antibodies binding to the lacto-ceramide core Galb(1,4)Glcb(1-1¢)Cer. Binding to this minimum building block can be capitalized on for cancer therapy and diagnostics and illustrates a new approach in designing cancer vaccines taking advantage of the latent polyspecificity of antibodies and the relevance of natural antibodies in antigen discovery and design.

18. Pashov, A., V. Shivarov, M. Hadzhieva, V. Kostov, D. Ferdinandov, K.-M. Heintz, S. Pashova, M. Todorova, T. Vassilev, T. Kieber-Emmons, L. A. Meza-Zepeda, E. Hovig. Diagnostic Profiling of the Human Public IgM Repertoire with Scalable Mimotope Libraries. Frontiers in Immunology 10 (2019) (2796),1-14.

Specific antibody reactivities are routinely used as biomarkers, but the antibody repertoire reactivity (igome) profiles are still neglected. Here, we propose rationally designed peptide arrays as efficient probes for these system level biomarkers. Most IgM antibodies are characterized by few somatic mutations, polyspecificity, and physiological autoreactivity with housekeeping function. Previously, probing this repertoire with a set of immunodominant self-proteins provided a coarse analysis of the respective repertoire profiles. In contrast, here, we describe the generation of a peptide mimotope library that reflects the common IgM repertoire of 10,000 healthy donors. In addition, an appropriately sized subset of this quasi-complete mimotope library was further designed as a potential diagnostic tool.

A 7-mer random peptide phage display library was panned on pooled human IgM. Next-generation sequencing of the selected phage yielded 224,087 sequences, which clustered in 790 sequence clusters. A set of 594 mimotopes, representative of the most significant sequence clusters, was shown to probe symmetrically the space of IgM reactivities in patients' sera. This set of mimotopes can be easily scaled including a greater proportion of the mimotope library. The trade-off between the array size and the resolution can be explored while preserving the symmetric sampling of the mimotope sequence and reactivity spaces. BLAST search of the non-redundant protein database with the mimotopes sequences yielded significantly more immunoglobulin J region hits than random peptides, indicating a considerable idiotypic connectivity of the targeted igome. The proof of principle predictors for random diagnoses was represented by profiles of mimotopes. The number of potential reactivity profiles that can be extracted from this library is estimated at more than 10⁷⁰. Thus, a quasi-complete IgM mimotope library and a scalable representative subset thereof are found to address very efficiently the dynamic diversity of the human public IgM repertoire, providing informationally dense and structurally interpretable IgM reactivity profiles.

19. Pashov, A. D.; Dimitrov, J. D., Antibody Polyreactivity: A Challenger of Immune Paradigms. Immunology 2025, 176, (4), 421-437.

Polyreactivity refers to the ability of antibodies to bind multiple unrelated antigens, encompassing polyspecificity and promiscuity. Here, we discuss the diverse molecular mechanisms underlying polyreactivity, including conformational dynamics, sequence- and structural characteristics of antigen-binding sites. The importance of polyreactive antibodies in immune defence and immune homeostasis is highlighted as well as their potential pathological consequences. Polyreactivity is seen as a continuum rather than a discrete property so that ultimately all antibodies possess some degree of polyreactivity. The challenges in defining antibody specificity are examined, and a shift towards quantitative thinking in antibody research is suggested. This would foster the adoption of novel methodologies to study complex antibody-antigen interactions at a systems level. Finally, the deeper understanding of polyreactivity's potential implications for the current antibody paradigm is critically evaluated.

20. Pashova, S., L. Balabanski, G. Elmadjian, A. Savov, E. Stoyanova, V. Shivarov, P. Petrov, A. Pashov. Restriction of the Global IgM Repertoire in Antiphospholipid Syndrome. Frontiers in Immunology 13 (2022).

The typical anti-phospholipid antibodies (APLA) in the anti-phospholipid syndrome (APS) are reactive with the phospholipid-binding protein β2GPI as well as a growing list of other protein targets. The relation of APLA to natural antibodies and the fuzzy set of autoantigens involved provoked us to study the changes in the IgM repertoire in APS. To this end, peptides selected by serum IgM from a 7-residue linear peptide phage display library (PDL) were deep sequenced. The analysis was aided by a novel formal representation of the Igome (the mimotope set reflecting the IgM specificities) in the form of a sequence graph. The study involved women with APLA and habitual abortions (n=24) compared to age-

matched clinically healthy pregnant women (n=20). Their pooled Igomes (297 028 mimotope sequences) were compared also to the global public repertoire Igome of pooled donor plasma IgM (n=2 796 484) and a set of 7-mer sequences found in the J regions of human immunoglobulins (n=4 433 252). The pooled Igome was represented as a graph connecting the sequences as similar as the mimotopes of the same monoclonal antibody. The criterion was based on previously published data. In the resulting graph, identifiable clusters of vertices were considered related to the footprints of overlapping antibody cross-reactivities. A subgraph based on the clusters with a significant differential expression of APS patients' mimotopes contained predominantly specificities underrepresented in APS. The differentially expressed IgM footprints showed also an increased cross-reactivity with immunoglobulin J regions. The specificities underexpressed in APS had a higher correlation with public specificities than those overexpressed. The APS associated specificities were strongly related also to the human peptidome with 1 072 mimotope sequences found in 7 519 human proteins. These regions were characterized by low complexity. Thus, the IgM repertoire of the APS patients was found to be characterized by a significant reduction of certain public specificities found in the healthy controls with targets representing low complexity linear self-epitopes homologous to human antibody J regions.

21. Pashova-Dimova, S.; Petrov, P.; Karachanak-Yankova, S.; Belezhanska, D.; Zhelev, Y.; Mehrabian, S.; Toncheva, D.; Traykov, L.; Pashov, A., Changes in the public IgM repertoire and its idiotypic connectivity in Alzheimer's disease and frontotemporal dementia. J Neuroimmunol 2025, 409, 578775.

Alzheimer's disease (AD) and frontotemporal dementia (FTD) are prevalent neurodegenerative disorders. Early diagnosis is challenging due to the lack of definitive biomarkers and reliance on invasive procedures. Immune biomarkers, particularly those reflecting the interaction between the central nervous system (CNS) and the peripheral immune system, have shown promise for non-invasive detection through blood samples. This study investigates the reactivity of serum IgM and IgG from AD and FTD patients against a library of mimotopes representing public IgM reactivities in healthy donors. Serum samples from AD, FTD, and other neurodegenerative dementias (ND) and controls were tested on peptide microarrays. The samples were pooled to mitigate individual variability. The reactivity data were analyzed using graphs to represent the cross-reactivity networks. The analysis revealed distinct reactivity patterns for the studied groups. Public IgM reactivities showed significant correlations with neurodegenerative conditions, with AD and FTD exhibiting loss or gain of specific IgM reactivities. Graph analysis highlighted significant differences between disease and control groups in graph density, clustering, and assortativity parameters. Mimotopes of IgM reactivities lost in dementia, particularly in AD, exhibited significant homology to HCDR3 sequences of human antibodies. Furthermore, clusters of reactivities showed significant distinctions between AD and FTD, with IgG reactivities providing additional differentiation. Several self-proteins related to neurodegeneration proved to have sequences homologous to disease-associated mimotopes. Interestingly, the beta-propeller signature sequence YWTD found in ApoE's receptor LRP1 proved a characteristic epitope for IgG in FTD but not AD. At the same time, the respective public gM mimotope YWTDSSR coincides with a highly conserved sequence in many microorganisms and sequences found in human HCDR3. Thus, the public IgM repertoire,

characterized by its broad reactivity and inherent autoreactivity, offers valuable insights into the immunological alterations in neurodegenerative diseases. The study supports the potential of IgM and IgG reactivity profiles as another compartment of non-invasive biomarkers for early diagnosis and differentiating AD and FTD.

22. Pashova-Dimova, S., P. Petrov, S. Karachanak-Yankova, **A. Pashov.** Neurodegenerative diseases associated antibody repertoire signatures in mimotope arrays based on cyclic versus linear peptides. Pharmacia 70 (2023) (4),1439-1447.

The role of peptide probes' conformational flexibility in extracting immunosignatures has not been sufficiently studied. Immunosignatures profile the antibody diversity and prove promising for early cancer detection and multi-disease diagnostics. A novel tool for modeling antibody repertoires, the concept of antibody reactivity graphs, proved instrumental in this respect. Serum samples from patients with Alzheimer's disease (AD), frontotemporal dementia (FTD), dementia of unknown etiology (DUE), and healthy controls were probed using a set of 130 7-mer peptides relevant to neurodegenerative diseases. Results show that linear peptides probed with IgM yielded higher graph density compared to IgG, indicating different levels of polyspecificities. Additionally, the impact of peptide topology and antibody isotype on feature selection was studied using recursive feature elimination. Findings reveal that IgM assays on linear peptides offer superior diagnostic differentiation of neurodegenerative diseases and define the degree of agreement between IgG and IgM immunosignatures with linear or cyclic peptides.

23. Pashova-Dimova, S., P. Petrov, A. Pashov. Igome Graphs Suggest the Changes in the IgM and IgG Repertoires in Antiphospholipid Syndrome Are in Part Idiotypically Defined. Proceedings of the Bulgarian Academy of Sciences 77 (2024) (11),1622–1628.

Antiphospholipid Syndrome (APS) is characterized by the persistence of high-affinity antiphospholipid antibodies, manifesting clinically as pregnancy complications, thrombosis, or neurological symptoms. This study explores the changes in the IgM and IgG antibody repertoires in APS patients using igome graphs, which represent antibody repertoires through peptide libraries selected from phage display on the complete immunoglobulin fraction of the serum. Serum samples from healthy women and APS patients were processed to isolate IgM and IgG fractions, which were then used for phage selection and next-generation sequencing. Peptide sequences were analyzed using graph representation, clustering, and spectral embedding to identify significant differences between the repertoires. The study revealed that while IgM repertoires in APS patients exhibit a loss of public reactivities, the IgG repertoires do not follow this trend. Three orthogonal sequence motifs were identified in the IgM and IgG repertoires, suggesting potential idiotypic interactions. These findings highlight the intricate mechanisms of antibody selection and cross-reactivity in APS, offering insights into potential therapeutic approaches. The study underscores the importance of system-level analysis in understanding immune

mechanisms and suggests that idiotypic interactions, though complex, could play a crucial role in APS pathogenesis.</p>

24. Planchais, C., J. Rayes, S. Delignat, S. Pashova, A. Varthaman, A. Pashov, J. Bayry, S. V. Kaveri, J. D. Dimitrov, S. Lacroix-Desmazes. Stimulation with FITC-labeled antigens confers B cells with regulatory properties. Cell Immunol 355 (2020) (104151),1-8.

B cells with regulatory properties (Bregs) were identified in human and in mice among different B-cell subsets. Their regulatory properties rely mainly on the production of anti-inflammatory cytokines, in particular IL10, IL-35 and TGFbeta, and were extensively studied in mouse models of autoimmune and inflammatory diseases. However, the exact nature of the stimulatory signals conferring regulatory properties to B cells is still not clear. We serendipitously observed that fluorescein isothiocyanate (FITC) binds to a significant proportion of naive mouse B cells. Binding of FITC to the B-cell surface implicated at least in part the B-cell receptor. It triggered IL-10 production and allowed the endocytosis of FITC-coupled antigens followed by their presentation to CD4(+) T cells. In particular, B cells incubated with FITC-OVA polarized OTII T cells towards a Tr1/Th2 phenotype in vitro. Further, the adoptive transfer of B cells incubated with FITC-labeled myelin oligodendrocyte glycoprotein peptide protected mice from experimental autoimmune encephalomyelitis, a T-cell-dependent autoimmune model. Together, the data show that FITC-stimulated B cells polarize immune responses towards Tr1/Th2 and acquire immuno-modulatory properties.

25. Saha, S., R. Murali, A. Pashov, T. Kieber-Emmons. The Potential Role of Solvation in Antibody Recognition of the Lewis Y Antigen. Monoclonal antibodies in immunodiagnosis and immunotherapy 34 (2015) (5),295-302.

Solvents play an important role in protein folding, protein-protein associations, stability, and specificity of recognition as in the case of antibody-antigen interactions through hydrogen bonds. One of the underappreciated features of protein-associated waters is that it weakens inter- and intra-molecular interactions by modulating electrostatic interactions and influencing conformational changes. Such observations demonstrate the direct relationship between macroscopic solvent effects on protein-protein interactions and atom-scale solvent-protein interactions. Although crystallographic solvents do explain some aspects of solvent-mediated interactions, molecular simulation allows the study of the dynamic role of solvents. Thus, analysis of conformations from molecular simulations are employed to understand the role of solvent on the inherent polyspecificity of a Lewis Y reactive germline gene relative to its expanded hybridomas and a humanized anti-Lewis Y antibody. Our analysis reveals that solvent mediates critical contacts through charged residues to facilitate cross-reactivity to carbohydrate antigens, but also increases the flexibility of some anti-Lewis Y antibodies concomitant with mutations (amino acid substitutions) to the germline antibody. Such flexibility might better allow for recognition and binding of internal structures of extended carbohydrate structures on tumor cells.

26. Saha, S., A. Pashov, E. R. Siegel, R. Murali, T. Kieber-Emmons. Defining the recognition elements of lewis y-reactive antibodies. PLoS ONE 9 (2014) (8),e104208.

Antibody response to carbohydrate antigens is often independent of T cells and the process of affinity/specificity improvement is considered strictly dependent on the germinal centers. Antibodies induced during a T cell-independent type 2 (TI-2) response are less variable and less functionally versatile than those induced with T cell help. The antigen specificity consequences of accumulation of somatic mutations in antibodies during TI-2 responses of Marginal Zone (MZ) B cells is a fact that still needs explanation. Germline genes that define carbohydrate-reactive antibodies are known to sculpt antibody-combining sites containing innate, key side-chain contacts that define the antigen recognition step. However, substitutions associated with MZ B cell derived antibodies might affect the mobility and polyspecificity of the antibody. To examine this hypothesis, we analyzed antibodies reactive with the neolactoseries antigen Lewis Y (LeY) to define the residue subset required for the reactive repertoire for the LeY antigen. Our molecular simulation studies of crystallographically determined and modeled antibody-LeY complexes suggests that the heavy-chain germline gene VH7183.a13.20 and the light-chain Vkappa cr1 germline gene are sufficient to account for the recognition of the trisaccharide-H determinant Types 1-4, while the specificity for LeY is driven by the CDR3 backbone conformation of the heavy chain and not the side chain interactions. These results confirm that these monoclonals use germline-encoded amino acids to recognize simple carbohydrate determinants like trisaccharide-H but relies on somatic mutations in the periphery of the combining site to modify affinity for LeY through electrostatic interactions that leads to their optimized binding. These observations bring further attention to the role of mutations in T-cell independent antibodies to distinguish self from non-self carbohydrate antigens.

27. Schneider, C., D. F. Smith, R. D. Cummings, K. F. Boligan, R. G. Hamilton, B. S. Bochner, S. Miescher, H.-U. Simon, A. Pashov, T. Vassilev, S. von Gunten. The human IgG anti-carbohydrate repertoire exhibits a universal architecture and contains specificity for microbial attachment sites. Science Translational Medicine 7 (2015) (269),269ra261.

Despite the paradigm that carbohydrates are T cell–independent antigens, isotype-switched glycan-specific immunoglobulin G (IgG) antibodies and polysaccharide-specific T cells are found in humans. We used a systems-level approach combined with glycan array technology to decipher the repertoire of carbohydrate-specific IgG antibodies in intravenous and subcutaneous immunoglobulin preparations. A strikingly universal architecture of this repertoire with modular organization among different donor populations revealed an association between immunogenicity or tolerance and particular structural features of glycans. Antibodies were identified with specificity not only for microbial antigens but also for a broad spectrum of host glycans that serve as attachment sites for viral and bacterial pathogens and/or exotoxins. Tumor-associated carbohydrate antigens were differentially detected by IgG antibodies, whereas non-IgG2 reactivity was predominantly absent. Our study highlights the power of systems biology approaches to analyze immune responses and reveals potential glycan antigen determinants that are relevant to vaccine design, diagnostic assays, and antibody-based therapies.

28. Shivarov, V., P. K. Petrov, A. D. Pashov. Potential SARS-CoV-2 Preimmune IgM Epitopes. Frontiers in Immunology 11 (2020) (932) .

While studying the human public IgM igome as represented by a library of 224,087 linear mimotopes, three exact matches to peptides in the proteins of SARS-CoV-2 were found: two in the open reading frame 1ab and one in the spike protein. Joining the efforts to fast track SARS-CoV-2 vaccine development, here we describe briefly these potential epitopes in comparison to mimotopes representing peptides of SARS-CoV, HCoV 229E and OC43.

29. Toshkova, N., V. Zhelyzkova, K. Koseva, K. Dimitrova, F. Elshaer, R. V. Lacombe, M. Lecerf, A. Pashov, J. D. Dimitrov. An integrative approach for profiling antibody responses in bats to human pathogens. EMI: Animal & Environment (2025),1-25.

Serological analyses are a fundamental tool for identifying infections by a wide range of pathogens. They offer a current overview of pathogen prevalence and insight into past infections. This is particularly relevant for bats, given their high capacity to tolerate pathogens and their role as reservoirs of zoonotic pathogens. At present, serological studies in bats have predominantly employed traditional techniques such as enzyme-linked immunosorbent assay (ELISA). However, these techniques have several limitations, including low throughput and the lack of bat-specific detection antibodies. To address these limitations, we developed an integrative approach for systemic serological analyses based on microarray technology, which enables the simultaneous detection of bat IgG antibodies against >190 human pathogens (viruses, bacteria, protists). The results of our analyses demonstrated an antibody response in bats targeting multiple epitopes from different pathogens, thereby proving the method's high-throughput capability. Furthermore, this approach does not rely on the use of IgG detection reagents, thereby allowing for its application to a diverse range of bat species. This assay offers insights into the infections of bats with pathogens, thereby enhancing our comprehension of zoonotic pathogens dynamics and facilitating targeted pathogen surveillance.

30. Bayry, J., A. Pashov, V. Donkova, S. Delignat, T. Vassilev, D. Stahl, B. Bellon, M. Kazatchkine, S. Lacroix-Desmazes, S. Kaveri. Immunomodulation of autoimmunity by intravenous immunoglobulin through interaction with immune networks. Vox Sang 83 (2002) (Suppl. 1),049-052.

Intravenous immunoglobulin (IVIg) has been used in the treatment of primary and secondary antibody deficiencies for over 25 years. IVIg was first demonstrated to be effective in autoimmune disorders two decades ago in the treatment of acute immune thrombocytopenia. Since then, the therapeutic efficacy of IVIg has been established in the Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, myasthenia gravis, dermatomyositis, Kawasaki syndrome and the prevention of graft

versus host disease in recipients of allogeneic bone marrow transplants, and reported in a large number of other autoimmune and systemic inflammatory conditions:

Immune-mediated diseases in which a beneficial effect of /V/g has been reported:
Idiopathic Thrombocytopenic Purpura (ITP)* Acquired Immune Thrombocytopenias Autoimmune Neutropenia Autoimmune Hemolytic Anemia Autoimmune Erythroblastopenia Parvovirus BlPassociated red cell aplasia
Anti-factor VIII autoimmune disease, Acquired von Willebrand's disease
Guillain-Barré Syndrome* Chronic Inflammatory Demyelinating Polyneuropathy
Myasthenia Gravis* Multifocal neuropathy (CIDP)*
Polymyositis Dermatomyositis
Kawasaki Disease* ANCA-positive systemic vasculitis Antiphospholipid syndrome Recurrent spontaneous abortions Rheumatoid Arthritis and Felty's syndrome JRA SLE
Thyroid ophthalmopathy, Birdshot retinochoroidopathy* Graft Versus Host Disease*
Multiple Sclerosis Insulin-Dependent Diabetes Mellitus
Steroid-dependent asthma Steroid-dependent severe atopic dermatitis Crohn's disease

* indicates diseases in which evidence for the effect of IVIg has been obtained in controlled trials.

31. Kieber-Emmons, T. and A. Pashov (2022). "Living with Endemic COVID-19." Monoclon Antib Immunodiagn Immunother **41**(4): 171-172.

COVID-19 vaccine performance has been difficult to define because outcomes were framed across four distinct goals: preventing infection, severe disease and death, hospitalization, and onward transmission. Public policy often emphasized "transmissible viral load," driving debate over masking, self-testing, and isolation duration. Yet the first widely deployed vaccines primarily elicited antibodies to the SARS-CoV-2 SPIKE protein, aiming to limit illness by interfering with viral entry via ACE2 rather than reliably halting transmission. The relationship among ACE2 expression or polymorphisms, infectivity, and disease severity remains incompletely understood, and the emergence of variants has underscored the limits of spike-focused immunity. Successive waves (Alpha, Delta, Omicron) illustrate that increased transmissibility plus immune escape can sustain symptomatic infections even in highly vaccinated populations, suggesting eradication is unlikely. Protection against severe outcomes is evident, but its duration is uncertain, and reinfections—including after prior infection—highlight constraints of acquired immunity alone.

As SARS-CoV-2 becomes **endemic**, strategies centered on universal self-testing and voluntary self-isolation may offer diminishing returns given substantial asymptomatic spread. Future management may resemble influenza: periodic boosting, continued pursuit of pan-coronavirus and nasal vaccines, and rapid variant-updated manufacturing. The commentary calls for quantitative models comparing SARS-CoV-2 and influenza evolutionary dynamics, and for renewed emphasis on antivirals to reduce replication, transmission, and the probability of new variants—especially by limiting spread in immunocompromised hosts.

32. Pashov, A. and T. Kieber-Emmons (2021). "Will a B1.1.1.529 Vaccine Be Undermined by Antigenic Sin: An Idiotypy Inspired Workaround." Monoclon Antib Immunodiagn Immunother **40**(6): 237-238.

A highly divergent SARS-CoV-2 variant, B.1.1.529 (Omicron), has drawn urgent attention due to extensive spike evolution, including numerous mutations within the receptor-binding domain, and early reports suggesting rapid spread. If antigenic distance from prior variants is large, the main risk may extend beyond reduced pre-existing immunity to misdirected immunity driven by original antigenic sin (OAS) and antigenic seniority. In these phenomena, recall responses to conserved epitopes can suppress de novo responses to newly mutated epitopes, potentially leaving individuals with prior infection- or vaccine-induced memory less able to mount effective neutralizing responses against neoepitopes. In a worst-case scenario, if only a few neutralizing epitopes remain conserved and cross-reactivity is insufficient, OAS could lead to more severe disease in previously immune individuals than in immunologically naive hosts.

To mitigate this, a vaccine design that presents mutated neutralizing neoepitopes while minimizing exposure to known recall epitopes is proposed. One strategy is to graft selected neoepitope peptides into immunoglobulin complementarity-determining regions, exploiting the relative “blind spot” of self-Ig frameworks, and deliver these chimeric antibodies via an mRNA platform, potentially with added pathogen-derived T cell epitopes. After priming naive B cell clones, boosters could revert to full-length antigen. Strong adjuvants (e.g., CpG) may help but do not directly eliminate recall-driven competition. OAS in COVID-19 remains plausible but unproven.