Capsule

Genetic variation in the serotonin receptor gene affects immune responses in rheumatoid arthritis

Many genetic variants associate with the risk of developing rheumatoid arthritis (RA); however, their functional roles are largely unknown. Snir and colleagues investigated whether the RA-associated serotonin receptor 2A (*HTR2A*) haplotype affects T cell and monocyte functions. Patients with established RA (n=379) were genotyped for two single-nucleotide polymorphisms in the *HTR2A* locus, rs6314 and rs1328674, to define the presence of the risk haplotype for each individual. Patients with and without the RA-associated TC haplotype were selected and T cell and monocyte function was monitored following in vitro stimulations with staphylococcal enterotoxin B and lipopolysaccharide using multiparameter flow cytometry. Within the cohort, 44 patients were heterozygous for the TC haplotype (11.6%) while none were homozygous. Upon stimulation, T cells from TC-carrier patients produced more pro-inflammatory cytokines, namely tumor necrosis factor-alpha (TNFα), interleukin-17 and interferon gamma, and monocytes produced higher levels of TNFα compared with patients carrying the non-TC haplotype ($P < 0.05$ and 0.01, respectively). Such cytokine production could be inhibited in the presence of the selective 5-HT2 receptor agonist (2,5-dimethoxy-4-iodoamphetamine, DOI); interestingly, this effect was more pronounced in TC carriers. Our data demonstrate that association of RA with a distinct serotonin receptor haplotype has functional impact by affecting the immunological phenotype of T cells and monocytes.

Genes Immunity 2013; 14: 83

Eitan Israeli

Capsule

The origin of the plague

The Justinian Plague, which resurfaced regularly between the 6th and 8th centuries, is thought to have assisted the decline of the Roman Empire, but it has, until now, only been speculatively diagnosed as bubonic plague caused by the bacterium *Yersinia pestis*. Using stringent ancient DNA anti-contamination protocols, Harbeck et al. have genotyped new material from the early medieval graveyard at Aschheim, Bavaria, dating from the 6th century. This graveyard contained 438 individuals, often in multiple burials – a sign of crisis. The amount of bacterial material available was scant, but *Y. pestis* was identified from one individual using five key single-nucleotide polymorphisms identified in recent phylogenies. Genotyping confirmed this isolate as basal to isolates from the 14th century Black Death and the modern (19th century) third pandemic and that, like the other pandemics, it originated in China or Mongolia.

PLoS Pathogens 2013; 9: 10.1371/journal.ppat.1003349

Eitan Israeli

Capsule

Receptor binding by a ferret-transmissible H5 avian influenza virus

Cell surface-receptor binding by influenza viruses is a key determinant of their transmissibility, both from avian and animal species to humans as well as from human to human. Highly pathogenic avian H5N1 viruses that are a threat to public health have been observed to acquire affinity for human receptors, and transmissible-mutant-selection experiments have identified a virus that is transmissible in ferrets, the generally accepted experimental model for influenza in humans. Xiong and collaborators showed that quantitative biophysical measurements of the receptor-binding properties of hemagglutinin (HA) from the transmissible mutant indicate a small increase in affinity for human receptor and a marked decrease in affinity for avian receptor. From analysis of virus and HA binding data the authors have derived an algorithm that predicts virus avidity from the affinity of individual HA-receptor interactions. It reveals that the transmissible mutant virus has a 200-fold preference for binding human over avian receptors. The crystal structure of the transmissible mutant HA in complex with receptor analogues shows that it has acquired the ability to bind human receptor in the same folded-back conformation as seen for HA from the 1918, 1957, 1968 and 2009 pandemic viruses. This binding mode is substantially different from that by which non-transmissible wild-type H5 virus HA binds human receptor. The structure of the complex also explains how the change in preference from avian to human receptors arises from the Gln226Leu substitution, which facilitates binding to human receptor but restricts binding to avian receptor. Both features probably contribute to the acquisition of transmissibility by this mutant virus.

Nature 2013; 497: 392

Eitan Israeli

Capsule

Innate lymphoid cells regulate CD4+ T cell responses to intestinal commensal bacteria

Innate lymphoid cells (ILCs) are a recently characterized family of immune cells that have critical roles in cytokine-mediated regulation of intestinal epithelial cell barrier integrity. Alterations in ILC responses are associated with multiple chronic human diseases, including inflammatory bowel disease, implicating a role for ILCs in disease pathogenesis. Owing to an inability to target ILCs selectively, experimental studies assessing ILC function have predominantly used mice lacking adaptive immune cells. However, in lymphocyte-sufficient hosts ILCs are vastly outnumbered by CD4+ T cells, which express similar profiles of effector cytokines. Therefore, the function of ILCs in the presence of adaptive immunity and their potential to influence adaptive immune cell responses remain unknown. To test this, Hepworth and group used genetic or antibody-mediated depletion strategies to target murine ILCs in the presence of an adaptive immune system. The authors show that loss of retinoic-acid-receptor-related orphan receptor-γt-positive (RORγt+) ILCs was associated with dysregulated adaptive immune cell responses against commensal bacteria and low-grade systemic inflammation. Remarkably, ILC-mediated regulation of adaptive immune cells occurred independently of interleukin (IL)-17A, IL-22 or IL-23. Genome-wide transcriptional profiling and functional analyses revealed that RORγt+ ILCs express major histocompatibility complex class II (MHCII) and can process and present antigen. However, rather than inducing T cell proliferation, ILCs acted to limit commensal bacteria-specific CD4+ T cell responses. Consistent with this, selective deletion of MHCII in murine RORγt+ ILCs resulted in dysregulated commensal bacteria-dependent CD4+ T cell responses that promoted spontaneous intestinal inflammation. These data confirm that ILCs maintain intestinal homeostasis through MHCII-dependent interactions with CD4+ T cells that limit pathological adaptive immune cell responses to commensal bacteria.

Nature 2013; 498: 113

Eitan Israeli

Capsule

Control of angiogenesis by AIBP-mediated cholesterol efflux

Cholesterol is a structural component of the cell and is indispensable for normal cellular function, although its excess often leads to abnormal proliferation, migration, inflammatory responses and/or cell death. To prevent cholesterol overload, ATP-binding cassette (ABC) transporters mediate cholesterol efflux from the cells to apolipoprotein A-I (apoA-I) and the apoA-I-containing high density lipoprotein (HDL). Maintaining efficient cholesterol efflux is essential for normal cellular function. However, the role of cholesterol efflux in angiogenesis and the identity of its local regulators are poorly understood. Fang et al. show that apoA-I binding protein (AIBP) accelerates cholesterol efflux from endothelial cells to HDL and thereby regulates angiogenesis. AIBP- and HDL-mediated cholesterol depletion reduces lipid rafts, interferes with VEGFR2 (also known as KDR) dimerization and signaling and inhibits vascular endothelial growth factor-induced angiogenesis in vitro and mouse aortic neovascularization ex vivo. Notably, Aibp, a zebrafish homologue of human AIBP, regulates the membrane lipid order in embryonic zebrafish vasculature and functions as a non-cell-autonomous regulator of angiogenesis. *aibp* knockdown results in dysregulated sprouting/branching angiogenesis, whereas forced Aibp expression inhibits angiogenesis. Dysregulated angiogenesis is phenocopied in Abca1 (also known as Abca1a) Abcg1-deficient embryos, and cholesterol levels are increased in Aibp-deficient and Abca1 Abcg1-deficient embryos. Our findings demonstrate that secreted AIBP positively regulates cholesterol efflux from endothelial cells and that effective cholesterol efflux is critical for proper angiogenesis.

Nature 2013; 498: 118

Eitan Israeli

Capsule

Wolbachia invades *Anopheles stephensi* populations and induces refractoriness to Plasmodium infection

Wolbachia is a maternally transmitted symbiotic bacterium of insects that has been proposed as a potential agent for the control of insect-transmitted diseases. One of the major limitations preventing the development of Wolbachia for malaria control has been the inability to establish inherited infections of Wolbachia in anopheline mosquitoes. Bian et al. report the establishment of a stable Wolbachia infection in an important malaria vector, *Anopheles stephensi*. In *A. stephensi*, Wolbachia strain wAlbB displays both perfect maternal transmission and the ability to induce high levels of cytoplasmic incompatibility. Seeding of naturally uninfected *A. stephensi* populations with infected females repeatedly resulted in Wolbachia invasion of laboratory mosquito populations. Furthermore, wAlbB conferred resistance in the mosquito to the human malaria parasite *Plasmodium falciparum*.

Science 2013; 340: 748

Eitan Israeli

Capsule

Compounds that interfere with KRAS localization have anticancer activity

The discovery that the mutational activation of RAS proteins drives the growth of human cancer cells catalyzed a dogged – but ultimately unsuccessful – search for drugs that inhibit RAS activity. Interest in pharmacologically targeting RAS has been revived by cancer genome studies, which revealed KRAS to be the most frequently mutated gene in the cancer types that are most common in the population and/or most refractory to therapy, such as pancreatic, lung, and colorectal cancer. Because KRAS signaling activity is dependent on the protein's localization at the cell membrane, Zimmermann and colleagues investigated whether compounds that interfere with KRAS localization have anticancer activity. In a high-throughput screen, they identified small molecules that prevent KRAS from binding to PDE-δ, a protein that facilitates KRAS trafficking to the membrane. An optimized compound, deltarasin, was found to inhibit KRAS signaling and growth of KRAS-mutant human pancreatic cancer cells in vitro and in mice. Although these results are promising, the bar for deltarasin and its derivatives will be high, because previous drugs designed to disrupt KRAS membrane localization in a different way proved to be ineffective in clinical trials.

Nature 2013; 10.1038/nature12205

Eitan Israeli

Capsule

The shaping and functional consequences of the microRNA landscape in breast cancer

MicroRNAs (miRNAs) show differential expression across breast cancer subtypes, and have both oncogenic and tumor-suppressive roles. Dvinge and co-authors report the miRNA expression profiles of 1302 breast tumors with matching detailed clinical annotation, long-term follow-up and genomic and messenger RNA expression data. This provides a comprehensive overview of the quantity, distribution and variation of the miRNA population and provides information on the extent to which genomic, transcriptional and post-transcriptional events contribute to miRNA expression architecture, suggesting an important role for post-transcriptional regulation. The key clinical parameters and cellular pathways related to the miRNA landscape are characterized, revealing context-dependent interactions, for example with regards to cell adhesion and Wnt signaling. Notably, only prognostic miRNA signatures derived from breast tumors devoid of somatic copy-number aberrations (CNA-devoid) are consistently prognostic across several other subtypes and can be validated in external cohorts. The authors then use a data-driven approach to seek the effects of miRNAs associated with differential co-expression of mRNAs, and find that miRNAs act as modulators of mRNA-mRNA interactions rather than as on-off molecular switches. They demonstrate such an important modulatory role for miRNAs in the biology of CNA-devoid breast cancers, a common subtype in which the immune response is prominent. These findings represent a new framework for studying the biology of miRNAs in human breast cancer.

Nature 2013; 497: 378

Eitan Israeli

Capsule

Synthetic generation of influenza vaccine viruses for rapid response to pandemics

During the 2009 H1N1 influenza pandemic, vaccines for the virus became available in large quantities only after human infections peaked. To accelerate vaccine availability for future pandemics, Dormitzer et al. developed a synthetic approach that very rapidly generated vaccine viruses from sequence data. Beginning with hemagglutinin (HA) and neuraminidase (NA) gene sequences, the authors combined an enzymatic, cell-free gene assembly technique with enzymatic error correction to allow rapid, accurate gene synthesis. Then they used these synthetic HA and NA genes to transfect Madin-Darby canine kidney (MDCK) cells that were qualified for vaccine manufacture with viral RNA expression constructs encoding HA and NA and plasmid DNAs encoding viral backbone genes. Viruses for use in vaccines were rescued from these MDCK cells. The authors performed this rescue with improved vaccine virus backbones, increasing the yield of the essential vaccine antigen, HA. Generation of synthetic vaccine seeds, together with more efficient vaccine release assays, would accelerate responses to influenza pandemics through a system of instantaneous electronic data exchange followed by real-time, geographically dispersed vaccine production.

Sci Transl Med 2013: 5: 185

Eitan Israeli

Capsule

Gut metagenome in European women with normal, impaired and diabetic glucose control

Type 2 diabetes (T2D) is a result of complex gene-environment interactions, and several risk factors have been identified, including age, family history, diet, sedentary lifestyle and obesity. Statistical models that combine known risk factors for T2D can partly identify individuals at high risk of developing the disease. However, these studies have so far indicated that human genetics contributes little to the models, whereas socio-demographic and environmental factors have greater influence. Recent evidence suggests the importance of the gut microbiota as an environmental factor, and an altered gut microbiota has been linked to metabolic diseases including obesity, diabetes and cardiovascular disease. Karisson et al. used shotgun sequencing to characterize the fecal metagenome of 145 European women with normal, impaired or diabetic glucose control. The authors observed compositional and functional alterations in the metagenomes of women with T2D, and develop a mathematical model based on metagenomic profiles that identified T2D with high accuracy. They applied this model to women with impaired glucose tolerance and show that it can identify women who have a diabetes-like metabolism. Furthermore, glucose control and medication were unlikely to have major confounding effects. They also applied the model to a recently described Chinese cohort and show that the discriminant metagenomic markers for T2D differ between the European and Chinese cohorts. Therefore, metagenomic predictive tools for T2D should be specific for the age and geographical location of the populations studied.

Nature 2013; 498: 99

Eitan Israeli

Capsule

Chemotherapy-induced bone marrow nerve injury impairs hematopoietic regeneration

Anticancer chemotherapy drugs challenge hematopoietic tissues to regenerate but commonly produce long-term sequelae. Chemotherapy-induced deficits in hematopoietic stem or stromal cell function have been described, but the mechanisms mediating hematopoietic dysfunction remain unclear. Administration of multiple cycles of cisplatin chemotherapy causes substantial sensory neuropathy. Lucas et al. demonstrate that chemotherapy-induced nerve injury in the bone marrow of mice is a crucial lesion impairing hematopoietic regeneration. Using pharmacological and genetic models, the authors show that the selective loss of adrenergic innervation in the bone marrow alters its regeneration after genotoxic insult. Sympathetic nerves in the marrow promote the survival of constituents of the stem cell niche that initiate recovery. Neuroprotection by deletion of *Trp53* in sympathetic neurons or neuroregeneration by administration of 4-methylcatechol or glial-derived neurotrophic factor (GDNF) promotes hematopoietic recovery. These results demonstrate the potential benefit of adrenergic nerve protection for shielding hematopoietic niches from injury

Nature Med 2013; 19: 695

Eitan Israeli

Capsule

Negligible impact of rare autoimmune-locus coding-region variants on missing heritability

Genome-wide association studies (GWAS) have identified common variants of modest-effect size at hundreds of loci for common autoimmune diseases; however, a substantial fraction of heritability remains unexplained, to which rare variants may contribute. To discover rare variants and test them for association with a phenotype, most studies re-sequence a small initial sample size and then genotype the discovered variants in a larger sample set. This approach fails to analyze a large fraction of the rare variants present in the entire sample set. Hunt et al. performed simultaneous amplicon-sequencing-based variant discovery and genotyping for coding exons of 25 GWAS risk genes in 41,911 UK residents of white European origin, comprising 24,892 subjects with six autoimmune disease phenotypes and 17,019 controls. They showed that rare coding-region variants at known loci have a negligible role in common autoimmune disease susceptibility. These results do not support the rare-variant synthetic genome-wide association hypothesis (in which unobserved rare causal variants lead to association detected at common tag variants). Many known autoimmune disease risk loci contain multiple, independently associated, common and low-frequency variants, and so genes at these loci are a priori stronger candidates for harboring rare coding-region variants than other genes. These data indicate that the missing heritability for common autoimmune diseases may not be attributable to the rare coding-region variant portion of the allelic spectrum, but perhaps, as others have proposed, may be a result of many common-variant loci of weak effect.

Nature 2013; 498: 232

Eitan Israeli

Capsule

Delaying embryo implantation in mammals

Many mammals can delay embryo implantation in order to postpone pregnancy when conditions are unfavorable, or until later birthing seasons. Such embryonic diapause occurs when development is suspended in the blastocyst stage and implantation is prevented. Endocrine factors trigger diapause, but the mechanism coordinating blastocyst dormancy and uterine quiescence remains unknown. Cha et al. show that the gene *Msx1* is expressed when implantation is delayed, whether it occurs because of maternal lactation, ovariectomy, or the addition of antiestrogen. When implantation initiates, *Msx1* expression is down-regulated. Further, genetic inactivation of *Msx1* or *Msx2* in the uterus results in the development of fewer blastocysts. In order for delayed implantation to occur, blastocyst dormancy must coincide with uterine quiescence. This work demonstrates a critical role of *Msx1* in maternal regulation of embryonic diapause. The study finds that three distantly related mammalian orders – Rodentia (mouse), Carnivora (American mink), and Diprotodontia (Australian tammar wallaby) – display correlations between Msx expression and diapause, suggesting the presence of a conserved reproductive strategy across mammalian species.

Open Biol 2013; 3: 10.1098/rsob.130035

Eitan Israeli

Capsule

Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis

Intestinal microbiota metabolism of choline and phosphatidylcholine produces trimethylamine (TMA), which is further metabolized to a pro-atherogenic species, trimethylamine-*N*-oxide (TMAO). Koeth et al. demonstrate that metabolism by intestinal microbiota of dietary l-carnitine, a trimethylamine abundant in red meat, also produces TMAO and accelerates atherosclerosis in mice. Omnivorous human subjects produced more TMAO than did vegans or vegetarians following ingestion of l-carnitine through a microbiota-dependent mechanism. The presence of specific bacterial taxa in human feces was associated with both plasma TMAO concentration and dietary status. Plasma l-carnitine levels in subjects undergoing cardiac evaluation (n=2595) predicted increased risks for both prevalent cardiovascular disease (CVD) and incident major adverse cardiac events (myocardial infarction, stroke or death), but only among subjects with concurrently high TMAO levels. Chronic dietary l-carnitine supplementation in mice altered cecal microbial composition, markedly enhanced synthesis of TMA and TMAO, and increased atherosclerosis, but this did not occur if intestinal microbiota was concurrently suppressed. In mice with an intact intestinal microbiota, dietary supplementation with TMAO or either carnitine or choline reduced in vivo reverse cholesterol transport. Intestinal microbiota may thus contribute to the well-established link between high levels of red meat consumption and CVD risk.

Nature Med 2013; 19: 576

Eitan Israeli

Capsule

Nonsense mutation in the *LGR4* gene is associated with several human diseases and other traits

Low bone mineral density (BMD) is used as a parameter of osteoporosis. Genome-wide association studies of BMD have hitherto focused on BMD as a quantitative trait, yielding common variants of small effects that contribute to the population diversity in BMD. Styrkarsdottir and team used BMD as a dichotomous trait, searching for variants that may have a direct effect on the risk of pathologically low BMD rather than on the regulation of BMD in the healthy population. Through whole-genome sequencing of Icelandic individuals, the authors found a rare nonsense mutation within the leucine-rich repeat-containing G-protein-coupled receptor 4 (*LGR4*) gene (c.376C>T), which is strongly associated with low BMD and with osteoporotic fractures. This mutation leads to termination of *LGR4* at position 126 and fully disrupts its function. The c.376C>T mutation is also associated with electrolyte imbalance, late onset of menarche and reduced testosterone levels, as well as an increased risk of squamous cell carcinoma of the skin and biliary tract cancer. Interestingly, the phenotype of carriers of the c.376C>T mutation overlaps that of Lgr4 mutant mice.

Nature 3023; 497: 517

Eitan Israeli

Capsule

The TLR4 antagonist Eritoran protects mice from lethal influenza infection

There is a pressing need to develop alternatives to annual influenza vaccines and antiviral agents licensed for mitigating influenza infection. Previous studies reported that acute lung injury caused by chemical or microbial insults is secondary to the generation of host-derived, oxidized phospholipid that potently stimulates Toll-like receptor 4 (TLR4)-dependent inflammation. Subsequently, Shirey et al. found that Tlr4–/– mice are highly refractory to influenza-induced lethality, and proposed that therapeutic antagonism of TLR4 signaling would protect against influenza-induced acute lung injury. Now they report that therapeutic administration of Eritoran (also known as E5564) – a potent, well-tolerated, synthetic TLR4 antagonist – blocks influenza-induced lethality in mice, as well as lung pathology, clinical symptoms, cytokine and oxidized phospholipid expression, and decreases viral titers. CD14 and TLR2 are also required for Eritoran-mediated protection, and CD14 directly binds Eritoran and inhibits ligand binding to MD2. Thus, Eritoran blockade of TLR signaling represents a novel therapeutic approach for inflammation associated with influenza, and possibly other infections.

Nature 2013; 497: 498

Eitan Israeli