OSPEDALE
SAN RAFFAELE

Services
Expertises
Research Tools
Animal Models

available for licensing, collaboration and business opportunities

Services

ALLTOX (www.alltox.it)
Burastero S.
Field: Allergology

Alltox is the joint venture between Abich, a company involved in analytical in vitro testing with special focus on biosafety issues, and the Laboratory of Cellular and Molecular Allergology of the Department of Biological and Technological Research at the San Raffaele Biomedical Science Park. Alltox provides state-of-the-art testing on the prediction of allergic sensitisation within the frame of current European regulatory directives. Alltox's unique expertise in in vitro testing allows us to investigate the sensitization potential of all finished cosmetic and toiletry products.
This is a strong advantage over the currently approved tests, which can be applied only to individual ingredients.
Abich provides a long-lasting expertise on biological and regulatory issues about biomedical and cosmetic products whereas the Laboratory of Cellular and Molecular Allergology has a documented experience on the cellular and molecular mechanisms of the generation of the allergic reaction in human experimental models.

Tests:
Sensitization
Cytokine release
Skin absorption
Nickel release
LLNA
Latex allergy

Toxicity analyses, immune modulation tests and evaluation of antiviral activity of Biological products

Malnati M.

Field: Toxicology

Biological products can be tested with the following validated assays:

1. Cellular toxicity assays are performed both on immortalised human cell lines and on human primary cell cultures, through cell counting and determination of viability with tripan blue or with tetrazolium salts (MTT).
2. Evaluation of immune modulatory activity is performed on human primary cell cultures i.e. lymphocytes, macrophages, or immature dendritic cells.
    - Limphocytes: are tested through the monitoring of the acquisition of triziate thymidine o through Elisa measurements of cytokines release that favour limphocyte growth
    - Macrophages: are tested through ELISA measurements of pro-inflammatory and/or immune modulant cytokines
    - Dendritic cells: are evaluated for phenotype differentiation and through ELISA measurements of pro-inflammatory and/or immune modulant cytokines

Valuation of antiviral activity. Monitoring of biological products activity on the in vitro growth of the immune deficiency virus (HIV-1) e Herpes Virus-6. Immortalised cell lines and primary human cell cultures are infected with the HIV or HPV-6 viruses and are subsequently morphologically evaluated and analysed through fluorescence microscopy and cyto-fluorimetry. The viral DNA concentration in the infected cultures is measured through a calibrated Real Time PCR.

Research Tools

Mouse Anti-Sars monoclonal antibody
Vicenzi E.
Field: SARS

CLONE: mAb8F4; ISOTYPE: IgG; IMMUNOGEN: Recombinant nuocleocapsid protein of SARS Co-V.
It has recently been shown that SARS is caused by a human coronavirus (SARS-CoV) (Ref. 1). SARS-CoV is a positive-stranded RNA virus, featuring the largest RNA genome known to date (29 kb). The first step in coronavirus infection is binding of the viral spike protein, a 139 kDa protein, to specific receptor(s) on host cells; in this regard, ACE-2 has been recently identified as a functional receptor for SARS Co-V (Ref. 3).The most prominent protein expressed in cells infected with SARS-CoV and released in the culture supernatants is the 46 kDa NC protein suggesting that NC is a potential major immunogen.
Our researchers have produced and tested two mouse monoclonal antibodies that recognize the severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) both in immunohistochemistry and Western blot as illustrated in more detail in the attached product description. To our knowledge these are the only monoclonal antibodies against SARS-CoV available on the market
APPLICATIONS: Immunofluorescence (IF), ELISA, Western blotting.

Mouse Anti-CD11b (LAK2) antibody
Zocchi M.

CLONE**:** 251F11
ISOTYPE**:** IgG1
IMMUNOGEN**:** Human cloned IL2-activated NK cells (LAK).

SPECIFICITY**:** A 140/110 surface protein expressed by human large granular lymphocytes (LGL), natural killer cells and monocytes. Clustered as an anti-human CD11b mAb at the Fourth International Workshop and Conference on Human Leukocyte Differentiation Antigens (Refs.2, 3).

APPLICATIONS: Immunofluorescence, hystochemistry, immunoprecipitation, western blot.

Human AntiERO1-Lalpha monoclonal antibody
R. Sitia

CLONE: mAb2G4
ISOTYPE: IgG
IMMUNOGEN: Maltose binding protein and human ERO1-Lalpha fusion protein
SPECIFICITY: For the maturation of newly synthesized proteins, it is the essential events in the ER to make the correct disulfide bonds. ERO1-Lalpha is one of the homologs of yeast Ero1p, which is essential for the oxidasation of cargo in the ER via PDI (Ref1-3).

Anti human-ERp44 monoclonal antibody
R. Sitia

CLONE: 6C9/39
ISOTYPE: IgG1k
IMMUNOGEN: GST and ERp44 fusion protein (Anelli et al., 2002)
SPECIFICITY: ERp44 is a protein of the early secretory pathway, which is involved in thiol-mediated retention of unassembled cargo-proteins (Anelli et al., 2002 and 2003)
APPLICATIONS: Immunoprecipitation, Western Blot (reducing and non reducing conditions, it recognizes also monkey but not murine ERp44) .

Anti human RanBPM monoclonal antibody.
R. Pardi

DESCRIPTION: RanBPM (RanBP9) Is a Phosphoprotein that associates with the Plasma Membrane and interacts with multiple membrane- expressed receptors.

IMMUNOGEN: antibodies against human RanBPM (RanBP9), aa 146-729 were cloned into pGEX4T1. The recombinant protein was purified on Glutathione-Sepharose, dialyzed against phosphate buffered saline (pH 7.4), and used to immunize mice (Balb/C). Anti-RanBPM antibodies were selected by their ability to recognize the antigen in ELISA assays,
and transfected RanBPM in western blots and in immunofluorescence assays.

CLONE: A subclone (SP1.177) of the original fusion, secreting IgG2a isotype anti-RanBPM Ab was eventually selected for high level of secretion and stability in culture.

ISOTYPE : IgG2a

SPECIFICITY: Consistently with the high degree of sequence conservation between murine and human RanBPM, our mAb recognized also the endogenous murine protein and detected RanBPM in all human and murine cell culture extracts and in all murine tissue lysates analyzed.

RESEARCH FIELD: cancer, biology of surface receptors, cell adhesion, integrines.

The product is for research use only. Not for pharmaceutical or drug use.

PCL12 Cell line
Scielzo C.
Field: Haematological disorders (CLL)

We recently document the establishment and characterization of a new cell line, PCL12 that grew spontaneously from the peripheral blood (PB) of a Chronic Lymphocytic Leukaemia (CLL) patient carrying EBV infection. Many efforts have been done to develop mouse models to study the disease pathogenesis but just few really representative cell lines are available, while this is an important tool to study CLL biology and test new treatments. PCL12 express CD19, CD5, CD20, CD23, sIgD, low levels of sIgM and the bad out-come prognostic markers CD38 (78%) and ZAP70 (37%). Accordingly to the bad phenotype IGV genes are un-mutated IGV and are aligned against the "original" clonotypic rearrangement from the patient in order to confirm its origin. BCR signalling pathway is constitutively active and anergic in terms of p-ERK and Calcium flux response, while is inactive in term of LYN and HS1 phosphorylation in accordance to the bad prognostic out-come.
PCL12 cells strongly migrate in response to SDF-1 and grow in clusters. Finally they grow very fast and localize all over in the CLL xenograft model Rag2-/-γc-/-, indicating that PCL12 represents a suitable preclinical model for testing pharmacological agents.

APPLICATIONS: tool to study in vitro and in vivo the disease and can be exploit to test new treatments.

Establishment and characterization of PCL12, a novel CD5+ Chronic Lymphocytic Leukaemia cell line.
Andreas Agathangelidis, Lydia Scarfò, Federica Barbaglio, Benedetta Apollonio, Maria Teresa Sabrina Bertilaccio, Pamela Ranghetti, Maurilio Ponzoni, Valeria De Pascali, Lorenza Pecciarini, Paolo Ghia, Federico Caligaris-Cappio and Cristina Scielzo.
PLoS One. 2015 Jun 25;10(6):e0130195.

Mouse models of diseases

1. Mouse EAN model:
Inventor: Quattrini A.
Field: Neuroscience

Experimental Autoimmune Neuritis (EAN) provides a useful model for understanding the mechanisms of acute and chronic inflammatory demyelinating polyradiculoneuropathy and new therapeutic strategies for human autoimmune neuropathies such as GBS and CIDP. EAN can be induced in susceptible strains by peripheral nervous system myelin or its components proteins with Freund's complete adjuvant (FCA). In rats EAN can be actively induced in susceptible strains by immunization with purified PNS myelin, bovine P2 protein, recombinant human P2 protein, or with peptides spanning the neuritogenic epitope of P2 protein. The main advantages of mouse EAN is that studies in genetically engineered mutants can be performed and less quantity of compounds is requested. EAN in mice is particularly useful in the C57BL/6 mice strains as a genetic background for transgenic and knockout animals. Histological analysis of EAN sciatic nerves show inflammation, demyelination and axonal damage in all mice and the pathology correlates with the clinical EAN score.

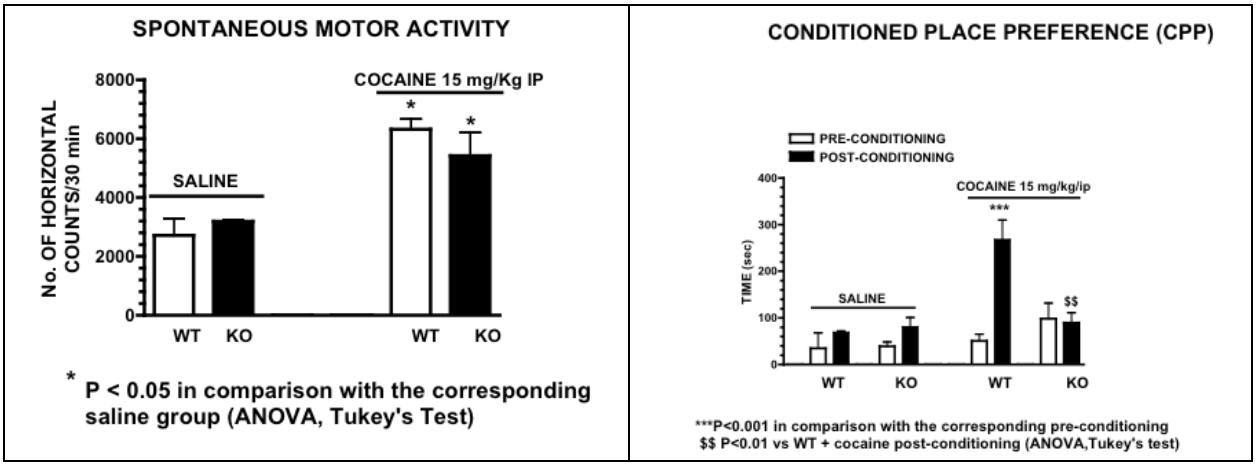
2. Mouse EAE model:
Inventor: Martino G.
Field: Neuroscience

We have generated a mouse model called Experimental Autoimmune Encephalomyelitis (EAE), characterised by demyelinization of the CNS. This model plays a central role in the Multiple Sclerosis (MS) research and in general in autoimmune diseases research.
Rat and mice induced EAE can be used to study:
- the immunopathological mechanisms responsible for the lost tolerance
against CNS antigens,
- the activation patterns of auto-reactive T lymphocytes with potential encephalitogenic and demyelinating activity,
- the trafficking of these lymphocytes and of other inflammatory cells through the emato-encefalic barrier,
- the effector mechanisms responsible for the demyelinization,
- the neuropathological aspect of these lesions and finally
- as a disease model to test new experimental therapies.

Besides the immunological studies described above, the EAE mouse model has been used to identify the genetic traits responsible for the disease insurgence or able to affect disease progression.

3. Mouse model for the study of addiction:
Inventor: Consalez G.
Field: Neuroscience

We have generated a genetically modified mouse strain wherein a particular gene, expressed in the ventral tegmental area of the midbrain and at various sites in the limbic system, has been deleted. The mouse displays a specific behavioral alteration, namely a defect in a test of conditioned place preference (CPP) after cocaine administration. The CPP test is an indicator of the reinforcing properties of cocaine or other psychoactive substances. Briefly, the test is conducted in an apparatus consisting of two compartments, one fitted with a grid floor and black walls (preferred by most mice under basal conditions) and the other with a mesh floor and white walls. The two are separated by a guillotine door. In the CPP test, mice of wild-type and mutant genotypes are allowed to explore the two compartments of the shuttle box for 15 min/day for 3 consecutive days (phase I, preconditioning period) and mice exhibiting an abnormal preference for the white compartment are discarded. In a subsequent conditioning period (phase II) lasting eight days, mice are taught to associate the dark compartment with saline administration, and the white compartment with cocaine administration. On the test day (phase III, postconditioning) neither drug nor vehicle is injected, and mice are individually placed at the intersection of the two compartments with access to both sides. The time spent in the white and black compartment is then measured over 15 min. Unlike their wildtype counterparts, in the CPP test our drug-treated mutants display no preference for the white compartment. Notably, the mutants display no significant defects in long term memory, as measured by the passive avoidance test. The same mutants respond to acute cocaine administration with an increase in spontaneous motor activity superimposable to that exhibited by their wild-type littermates. Thus, homozygous mutants display a selective disruption in the reinforcing response to cocaine administration. We believe that this mutant strain represents a very valuable model for the systematic analysis of molecular genetic cascades underlying cocaine addiction.



4. Mouse model for short term memory:
Inventor: Toniolo Daniela
Field: Neuroscience

The Gdi1 gene was identified in our laboratory as one of the genes responsible for human non-specific Mental Retardation (D'Adamo et al., Nat Gen 19, 134-139). A constitutive KO of the mouse Gdi1 gene was constructed. The mice were viable and fertile and did not present anatomical or histological defects. Behavioral analysis demonstrated that the mutant mice were less aggressive and presented defects in short term memory formation (D'Adamo et al., Hum Molec Genet 11, 2567-2580).
Further unpublished work, aimed at the characterization of the molecular defect(s) responsible for the memory deficit, is providing the molecular description of the synaptic alterations associated to memory loss, a very frequent cognitive disorder present in many forms of in Mental Retardation as well and in aging and dementia. Our data suggests that the Gdi1 KO mice represent a good and well characterized animal model to study the mechanisms of memory loss as well as to search for drugs and novel therapies.

5. TRAMP model to evaluate prostate cancer vaccination:
Inventor: Matteo Bellone
Field: Cancer

Prostate cancer (PC) is an emerging health problem in the more developed countries. With a population ageing rapidly, PC is one of the leading causes of death in men over 50. Traditional androgen ablation therapy, as well as surgery, chemotherapy and radiotherapy have not substantially reduced PC lethality. This is because prostatectomy and radiotherapy are potentially curative only for organ-confined diseases, and treatment of locally advanced or metastatic cancer remains only palliative.
The identification of prostate tumor-associated antigens (PTAA) has recently reinforced the efforts in pursuing active immunotherapy as complementary and/or alternative therapy for PC. Several vaccination strategies are actively pursed in PC and need to be tested and validated in realistic pre-clinical models.
Transgenic mice developing spontaneous tumors may overcome most of the limitations imposed by "classic" models of tumor cell implantation. Over the last 3-4 years we have been developing a deep and wide experience in the TRAMP (Transgenic Adenocarcinoma Mouse Prostate) model. These animals transgenic for the SV40 large antigen (Tag) and under the control of the rat probasin regulatory element, express Tag at puberty selectively on the prostate tissue. In the following weeks (wk) TRAMP mice invariably and progressively develop prostate intraepithelial neoplasia (PIN; wks 6-12), adenocarcinoma (wks10-16), and lymph node and lung metastases (wks 18-24), therefore resembling human PC. Furthermore in this model the PC expresses many of the PTAA commonly found in human PC and Tag behaves as a non-mutated PTAA, and can therefore be used as a model antigen.
We have correlated in TRAMP mice the disease progression, from health to death, with a fine dissection of the dynamics of the Tag-specific cytotoxic T lymphocyte response.
Our yet unpublished results suggest selected time windows for effective immune intervention. We are currently investigating in the TRAMP model several conventional and innovative combined therapeutic approaches for PC.
The developed expertise and know-how in the TRAMP model and vaccination strategies, the surrounding scientific community and network of collaborations, and the close proximity to the clinic (> 500 new PC patients/year) make at present our laboratory an ideal place were to rapidly test and validate any novel immunotherapeutic approach for PC.

6. Spontaneous mouse models of osteoporosis
Inventor: Simone Cenci
Field: Osteoporosis

Despite the huge impact of osteoporosis worldwide, our knowledge on bone genetics and biology remains remarkably incomplete. Animal models of bone loss are greatly needed for biotechnological and biomedical purposes.
We have established **two novel genetic mouse models that develop spontaneous, early-onset, severe osteoporosis**.
These strains develop almost complete loss of the bone trabecular architecture owing to excessive resorption, not compensated for by bone formation.
Besides recapitulating human osteoporosis, these alterations are ideal to test both antiresorptive and osteoanabolic drugs, against involutional, inflammatory or cancer-induced bone-wasting.

Contacts:

Paola Vella

Head, Office of Biotechnology Transfer
San Raffaele Hospital and Scientific Institute
Tel: +39 02 2643 4281
Fax: + 39 02 2643 8138
Email: vella.paola@hsr.it