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A STRONG AND RAPID IMMUNOSUPPRESSION BY EXTRACELLULAR ROS- CLEAVING ENZYMES (GSH-PEROXIDASE, CATALASE, MnSOD, CuZnSOD) GIVES EVIDENCE FOR A PERMANENT, O_2^- AND O_2^{2-}-MAINTAINED INTERCELLULAR CROSS TALK, EVEN IN THE RESTING STATE

ROS- CLEAVING ENZYMES AS NOVEL IMMUNOSUPPRESSANTS AND HO_2 AND H_2O_2 AS WELL AS NH_3 AND POLYAMINES AS NOVEL "SECOND MESSENGERS"

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Some observations supporting the novel hypothesis: (I) Both H_2O_2-cleaving enzymes GSH- peroxidase and catalase strongly suppress the activity of (a) resting (b) alloactivated, and (c) lectin-activated PBMCs. MnSOD and Cu/ ZnSOD also significantly inhibit PBMC-activity. The dependence of cell activity on the extracellular HO_2 and H_2O_2 even in resting cells gives evidence of a permanent cell cross-talk via ROS and allows the APCs(macrophages) to organize and coordinate the immune response (a) through a direct ROI secretion or (b) via post-modulation of secreted ROI by enzymes (catalase, GSH-POD, SOD). The activation of PBLs by HO_2 and H_2O_2, generated by xanthine oxidase (XOD)was so strong that it worked even in pure PBS, containing only Na and K ions. (II) We consider NH_3 as a novel „second messenger" which promotes proliferation (a) per se or (b) by synergizing with HO_2(and H_2O_2). The mitogenic mechanism is based on pH_i increase ($H^+ + NH_3 = NH_4^+$). NH_3 can be released by L-glutaminase or L-asparaginase and sequestered by L-glutamine (or asparagine) synthase. Since all oncogenes and growth factors increase pH_i by 0,2-0,3 pH units, and as NH_3 per se induces cell division, we consider HO_2 and NH_3 as late downstream mediator molecules of cell proliferation. Moreover, polyamines (putrescine), formed by proliferation-associated ornithine decarboxylase, contribute to the pH_i increase. The cytosolic increase of both pH_i and Ca_i support the mitochondrial Ca^{2+}/H^+ antiporter and here with the ROS generation.

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OUR NOVEL APPROACHES (A) IN THE PREVENTION OF THE ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE (GvHD) AND – EVEN MORE IMPORTANT – (B) IN THE ERADICATION OF PRE-ESTABLISHED GvHD COULD HELP TO INTRODUCE CLINICALLY A VARIETY OF ADOPTIVE IMMUNOTHERAPIES.

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The most important implications of our animal experiments are as follows:
(a) The replacement of untreated donor splenocytes by DNA-premanipulated, lifespan-predetermined MIS("MIS-BM/SC") effectors completely prevented GvHD and resulted in a dramatic improvement of the long-term survival rate (100% vs. 0%) both in the semi-syngeneic and fully allogeneic system.
(b) The eradication of the established GvHD is based on a radical elimination of alloreactive Tc cells of donor (donor I) origin through a combined treatment by chemo(radio)therapy, T(Tc)- depleting antibodies and the in vitro premanipulated, donor I-specific alloaggressive cytotoxic T cells of a further donor II, the so called 3rd party MIS-effectors ("MIS-GvH" effectors). The preconditioning of the patient suffering of GvHD can be carried out under non-ablative ("immunoablative") or myeloablative conditions. The efficiency of the "MIS-GvH" effectors can further be potentiated by the in vitro or in vivo prealloimmunization of donor II by donor I (whose T cells had induced the GvHD). An additional potentiation of the "MIS-GvH" effectors can be achieved by the vaccination of the donor II with hybrid cells, formed by the fusion of MHC II-positive cells (e.g. DC,B cells or macrophages of donor I with the APCs of donor II). An additional positive effect can be expected from the vaccination of the patient with a 2nd class of vaccines, based on cell hybrids of GvHD-inducing T cells with autologous (i.e. patient's) or allogeneic (donor I or II) HLA class II-positive (e.g. accessory) cells. Both types of the anti-GvHD vaccine are recommended especially in the case of the advanced or refractory GvHD.

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ESTABLISHMENT OF A STABLE MIXED ALLOGENEIC CHIMERISM BY THE SUCCESSIVE INJECTION OF A 1:1 MIXTURE OF MATURE, LIFESPAN-PREPROGRAMMED DONOR PLUS RECIPIENT T CELLS OR PBMCS, FOLLOWED BY A 1:1 MIXTURE OF IMMATURE, NON-DNA-PREMANIPULATED BONE MARROW OR STEM CELLS OF THE SAME DONOR : HOST ORIGIN

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The establishment of a stable mixed allogeneic chimerism (MACH) could be considered as one of the most urgent problems in adoptive immunotherapy.
One of the clinical situations where MACH plays a central role is the DLI treatment of relapsed bone marrow recipients; here, a preestablished MACH seems to modulate the GvM-accompanying GvHD towards a less aggressive form which may be restricted to the lymphohematologic system.
Since a prematurely established MACH coinduces tolerance against tumor cells, we consider an early MACH-induction rather as a contraproductive, GvM downregulating process and propose a changed clinical protocol, consisting of a transient fully allogeneic chimerism as the first step and MACH as the second step. In this way, the strong GvM effect, eradicating the MRD(minimal residual disease), is followed by a stable MACH which is able to prevent adverse side-effects, the GvHD(in BMT and HSCT) and the HvGR(in organ transplantation), respectively.
A further important clinical indication for a stable MACH are bone marrow-correctable immunodeficiencies and (other) genetic diseases. Here, a lifelong coexistence of donor and host cells, i.e. a permanent donor : host tolerance is necessary to provide the patient with donor-derived immunocompetent cells and – in the case of diverse, BMT treatable genetic diseases – with the gene defect-correcting (proficient) cells of a healthy donor.

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TWO IMPORTANT IMPROVEMENTS OF THE CONVENTIONAL BMT AND HSCT: (A) THE ROUTINE USE OF A 1:1 MIXTURE OF D- AND R- DERIVED BMCS (OR HSCS) PRECEDED BY THE 1:1 MIXTURE OF D- AND R- DERIVED MIS EFFECTORS, AND (B) THE ROUTINE USE OF HAPLOIDENTICAL MIS EFFECTORS, COMBINED WITH BMT OR HSCT

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The conventional allogeneic bone marrow and stem cell transplantation has the disadvantage that donor-type immunocytes continue to represent a kind of "foreign body". This results both in an impaired T cell : APC cooperation and in an affected recognition of tumor and viral structures on patient's own cells. Fine differences between tumor and normal cells can be recognized only if MHC I (and II) on tumor cells, APCs and thymic epithelial cells (responsible for MHC-restriction of T cells) are identical. In recipients of allogeneic bone marrow or stem cell grafts, the MHC-restriction of host thymus-educated T cells is different from the MHC structure on donor-derived accessory cells, so that the T cell : APC-cooperation is impaired. For this reason, we propose two improvements of existing techniques: (a) A routine use of a 1:1 mixture of donor plus recipient DNA-premanipulated mature T cells (or PBMCs), followed by the 1:1 mixture of the corresponding non-premanipulated immature bone marrow or stem cells. In this way, an optimized T cell : APC-cooperation can be restored, primarily due to the presence of the host-type APCs which are critical for the patient's immunocompetence against (retro)viral infections and malignancies. (b) The second improvement is the routine use of in vitro premanipulated MIS/MIT effectors, derived from haploidentical siblings, mimicking the above mentioned highly successful semi-allogeneic system (94,6% long-term survivors at a single treatment and a 100% long-term survival rate at a repeated treatment). Again, the central point is the appearance of common haplotype on the surface of the donor, the recipient and the transformed (or virally infected) target cells.