Human testis specifically expresses a homologue of the rodent T lymphocytes RT6 mRNA

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Abstract A human homologue of the rodent T cell mono ADP-ribosyl transferase RT6 mRNA was identified by a systematic analysis of human testis transcripts. This messenger encodes for a precursor protein of 367 aa (MW: 41.5 kDa) which exhibits a peptide signal, consensus domains for mono ADP-ribosyl transferase and a C-terminal part characteristic of glycophosphatidyl inositol anchored protein. This mRNA, transcribed from a gene localized in 4q13-q21, is not expressed in white blood cells but is specific for human testis in which it is likely to correspond to a new ADP-ribosyl transferase.

Key words: Human testis; Mono ADP-ribosyl transferase; RT6; Chromosome 4; Diabetes mellitus

1. Introduction

RT6 is a glycosylphosphatidyl inositol (GPI) anchored membrane protein [1,2] specifically expressed at the cell surface of rat and mouse T lymphocytes [2,3]. The rat has two alloantigens for this protein (rRT6.1 and rRT6.2) which both display a NAD-glycohydrolase activity [4,5], indicating that they are able to transfer ADP-ribose, but to a still unknown physiological acceptor.

Interestingly, it was shown that a defect in the $RT6^+$ lymphocyte population correlates with an increased susceptibility to insulin-dependent diabetes mellitus, in diabetes-prone Bio Breding rats (DPBB) and NOD mice [6,7]. This observation was strengthened by the fact that a transfusion of $RT6^+$ cells in DPBB rats prevented the outbreak of the disease, [8,9]. In addition, a similar defect in $RT6^+$ population exists in (NZW × NZB) mice which exhibit systemic lupus erythematosus, another autoimmune disease [10]. All these observations argue for a relationship between a decrease in $RT6^+$ T cell number and the susceptibility for autoimmune disease in rodent, even if the defect in RT6 expression is unlikely to be the primary event of the observed T cell lymphopenia [11–13].

Recently we characterized a large series of cDNAs from human testis by partial cDNA sequencing and by comparison of these sequences with nucleic and amino acid databases [14]. Using this strategy, we identified a clone (htMART) which exhibited a significant homology over 60 amino acids with the rat and mouse RT6 proteins. Due to the potential interest of this protein as a marker of T cells involved in autoimmune diseases, we decided to further characterize this clone. In this report, we demonstrate that in human the htMART mRNA is not expressed in white blood cells but is specific for testis in which it is likely to correspond to a new mono ADP-ribosyl transferase.

2. Materials and methods

The complete sequence of the htMART cDNA was determined, as previously described [14], at least three times on each strand of the insert. The Northern blot from rat and mouse tissues, as well as from human testis, was prepared as reported (5 μg of polyA+ mRNA per lane) [14] and Northern blots from different human tissues were obtained from Clontech (2 μg of polyA+ mRNA per lane). The "zoo blot" containing genomic DNA from human, monkey, rat, mouse, dog, cow, rabbit, chicken and yeast, was obtained from Clontech. The Southern blot of human genomic DNA was prepared by digestion of 40 μg of DNA using the restriction enzymes *Hin*dIII, *Pst*I, *Pvu*II, *Eco*RI and *Kpn*I and according to manufacturer's specifications. DNA fragments were resolved on a 0.8% agarose gel and transferred to nylon membrane (Hybond N). The blots were hybridized with the total htMART cDNA sequence labelled with a ^{32}P dCTP by random priming according to the Megaprime protocol (Amersham). The Southern blots were washed at 65°C in 1×SSC (0.15 M NaCl, 15 mM sodium citrate), 0.1% SDS (low stringency) or 0.1×SSC, 0.1% SDS (high stringency) and exposed for the indicated periods of time at −80°C on X-ray films between two screens (Kodak). The Northern blots were washed at 50°C in 0.1×SSC, 0.1% SDS (low stringency) or at 63°C in 0.05×SSC, 0.1% SDS (high stringency) and exposed as indicated for the Southern blots. Chromosomal localization was done by in situ hybridization as previously described [15]. The sequence alignments and comparisons were done using CLUSTALW, KSEQFP and KSEDF programs with their default parameters respectively (CITI2 server). The searches for homologous sequences were done either using the BLAST programs on the non-redundant nucleic acid and protein databases at the NCBI or the EMBL Fasta server.

3. Results

The complete nucleic acid sequence of the htMART cDNA (Genbank accession number: U47054) revealed a 5′ untranslated region of 31 bp, a 1101 bp open reading frame and a 3′ untranslated region of 278 bp. The predicted amino acids sequence (367 aa; MW: 41.5 kDa) is represented in Fig. 1. This sequence exhibits one potential glycosylation site (Asn^{248}) and three consensus motifs (Fig. 1 I, II and III) specific for enzymes catalyzing ADP ribose transfer [16]. Motif I includes a critical His^{94}; motif II is composed of 9 hydrophobic or aromatic residues; motif III contains two glutamate and several other acidic amino acids in the downstream region. Finally this sequence contains 3 repeated motives (GEKNQKLEDH) from G^{283} to H^{312} in its C-terminal region. Hydrophobic analysis according to Kyte and Doolitle [17] revealed a hydrophobic peptide signal from Phe^6 to Val^{24}, and a hydrophobic C-terminal part (Leu^{344} to Leu^{367}) compatible with a GPI anchored protein [18].

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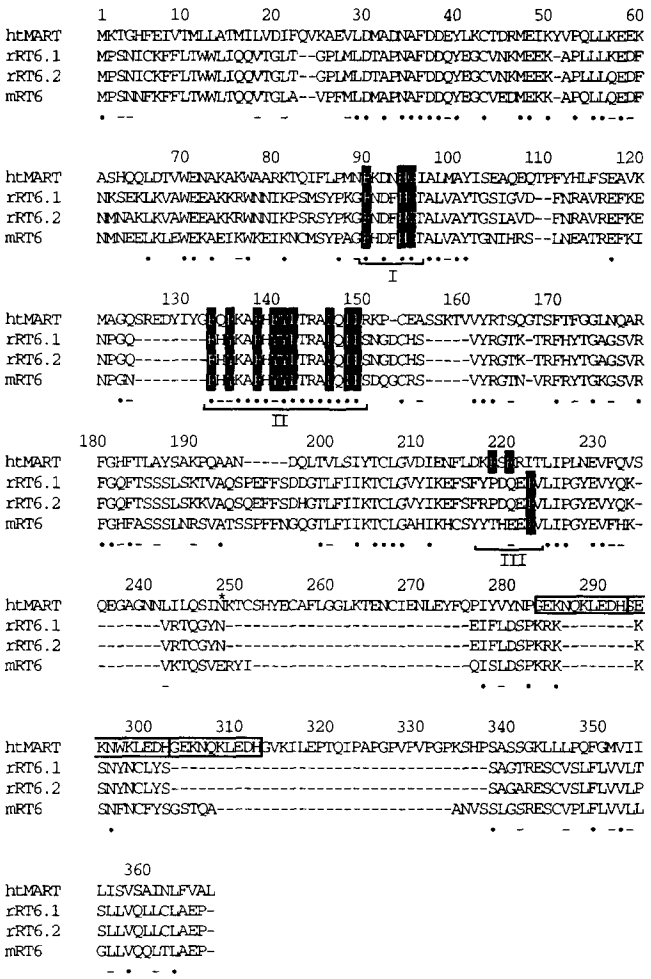

Fig. 1. Predicted amino acid sequence of htMART and its alignment with rat rRT6.1 [19], rRT6.2 [1] and mRT6 [20]. (●) and (–) indicate identical or similar residues among the four sequences. The conserved hydrophobic residues, corresponding to the three consensus regions (roman numerals) for mono ADP-ribosyl transferase, are in reverse-shaded letters. Asparagine^{250} (*) is the only possible *N*-glycosylation site in the human sequence. The three repeated blocks (G/S)EKNQKLEDH are boxed.

The alignments of htMART with the rat (rRT6.1 and rRT6.2) and mouse (mRT6) sequences are depicted in Fig. 1. htMART exhibits 35% and 57% of identities and similarities respectively over the whole length of the three rodent sequences. Among these identities, three cystein residues (Cys^{43}, Cys^{153}, Cys^{206}) are conserved and might be of importance for the secondary structure of the protein.

The expression of the htMART mRNA was analyzed by Northern blot of different human tissues. Under low stringency washing conditions, an abundant 1.8 kb messenger was detected in testis and skeletal muscle, whereas a weaker 1.6 kb signal was observed in heart (Fig. 2, A). At higher stringency, only the testis specific messenger remained detectable (Fig. 2, B). We used the same probe on a Northern blot of rat and mouse polyA+ mRNA from testis, as well as two mixtures of rat and mouse brain, liver, and kidney (Fig. 3). Even under low stringency washing conditions, we did not detect any cross hybridizing messenger with the htMART probe.

A Southern blot of human genomic DNA, cut by 5 restriction enzymes, was hybridized with the htMART cDNA probe. Under low stringency washing conditions, this probe revealed 1 to 4 bands per lane (Fig. 4) whereas only 1 or 2 bands were detected at higher stringency: 2 bands with *Hin*dIII* (9.5 kb, 1.8 kb), 2 with *Pst*I* (8.8 kb, 2.8 kb), 2 with *Eco*RI (8 kb, 1.5 kb), 2 with *Pvu*II (9.5 kb, 2.0 kb) and 1 with *Kpn*I (12 kb) (*cognate restriction sites in the probe). Such simple patterns are compatible with the presence of a single specific htMART gene. The same probe, hybridized to a "zoo blot" of genomic DNA cut by *Eco*RI, and washed under low stringency conditions, revealed specific bands on monkey (6.2 kb), dog (3 kb) and cow (6.7 kb), and no signal with the other species (Fig. 5). The band observed in monkey is similar in size to a band observed in *Eco*RI cut of human DNA hybridized under low stringency conditions (Fig. 4).

This gene was localized in the human genome. Out of the 150 metaphase cells examined following in situ hybridization, 223 silver grains were associated with chromosomes and 81 of these (36.3%) were located on chromosome 4. The distribution of grains on this chromosome was not random: 63/81 (77.7%) of them mapped to the q13-q21 region of chromosome 4 long arm (Fig. 6). These results allowed us to map the htMART gene to the 4q13-q21 bands of the human genome, in a region where seven genes have been already localized (amphiregulin, interleukine 8, annexin III, deoxycytidine kinase, glycerol kinase pseudogene 2, neutrophil-activating peptide ENA-78, Tyr04 protein tyrosine kinase).

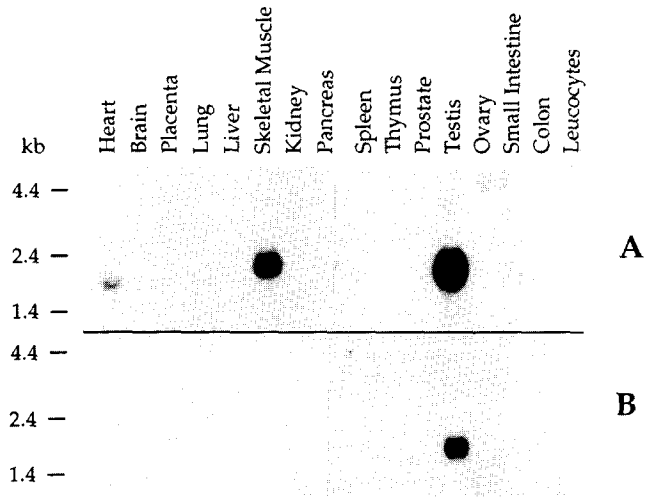

Fig. 2. Northern blots from human tissues mRNA hybridized with the htMART cDNA and washed under low (A) and high (B) stringency washing conditions. Exposures for A and B were 1 and 3 nights respectively. The size of the markers is indicated on the left hand side.

4. Discussion

The predicted htMART amino acid sequence that we have determined has several common features with the rat and mouse RT6 proteins. Firstly, the htMART sequence exhibits 35% identities and 57% of similarities with the whole length of the rodent RT6 amino acid sequence. This includes a very well conserved region, framed by the signal peptide and consensus region I of ADP ribosyl transferase; it corresponds to the initial hit indicating an homology between htMART and the rRT6 sequences. Secondly, based on the N and C terminal hydrophobic regions, htMART has the potential structure of a membranous GPI anchored protein [18] as observed for rat and mouse RT6 proteins [1,2]. Based on the alignment of htMART with the rodent sequences, the htMART signal peptide would include the aa 1 to 24 and the cleavage site of the C-terminal tail would be inside the Ser doublet 341–342. Thus the mature htMART would be 320 amino acids long. Thirdly, this sequence exhibits the three consensus regions specific for mono ADP-ribosyl transferase as observed for rodent RT6 proteins [16]. Region I contains the critical His^{94} which is known to play a role in hydrogen bonding. Region II is very well conserved and contains hydrophobic residues which are important for the positioning of the nicotinamide and adenine moieties. Region III has two specific features: (i) 2 Glu residues (Glu^{218} and Glu^{220}) are present at a slightly shifted position as compared to rat [19], mouse RT6 [20] and rabbit ADP ribosyl transferase [21] (i.e. at position 222 of the human sequence); (ii) 26 acidic residues in the region upstream the hydrophobic C-terminal end. These observations strongly argue for htMART being a new human ADP ribosyl transferase, but only its expression will determine whether it does exhibit such an enzymatic activity. Fourthly, htMART contains 6 Cys residues, of which the first three (Cys^{42}, Cys^{153} and Cys^{206}) are at the same position as in the rat and mouse sequences. The three others are in the downstream sequence, excluding the hydrophobic C-terminal part. Therefore htMART has conserved the potential to form at least two intrachain bonds as described for the rRT6.2 [1].

The most striking difference resides in the length of the amino acid sequences. htMART is 80 and 92 amino acids longer than rRT6 and mRT6 respectively. This additional stretch contains a motif (GEKNQKLEDH) repeated three times, with the exception of 2 amino acids changes in the central motif. Screening of sequences and motif databases did not provide any information on such a sequence and on its possible role in the protein function. These repeats did not result from a cloning artefact since the whole motif is present in the genomic DNA (Koch-Nolte and Thiele, personal communication).

In contrast, the tissue specific distribution of the human and rodent transcripts reveals their own specific features. Using the htMART cDNA as probe, we could establish the following points: (i) htMART transcript is specific for human testis; (ii) htMART is unlikely to be a marker of T-lymphocytes. Although T lymphocytes represent only 10% of the white blood cells, the use of polyA + mRNA is a favourable situation to detect a T cell specific messenger, at least of similar abundance of RT6 specific messenger present in T cell total RNA as used by others [22]. As a matter of fact, even under low stringency washing conditions, htMART cDNA did not detect any signal in human leukocytes mRNA; (iii) the rodent testis, as well as brain, liver and kidney, do not express a homologue of htMART.

Other groups have analyzed the RT6 expression in rat and mouse tissues, and have looked for cross-reactive messengers in different human tissues. They clearly established that: (i) RT6 is exclusively expressed in T lymphocyte and absent from rat testis [23]; (ii) a human genomic probe, having strong similarities with the rodent sequences (see further) [23], was unable to detect any transcript in the same human tissues as the ones analyzed in this study.

Altogether these data indicate that htMART and the rodent sequences belong to the mono ADP-ribosyl transferase family. However, their sites of expression are clearly different and

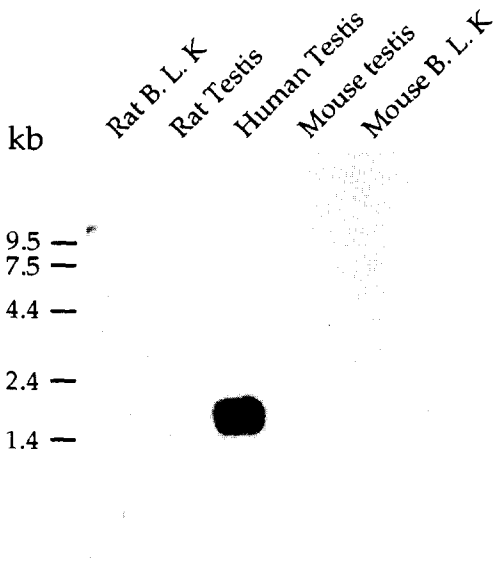

Fig. 3. Northern blot from rat, human and mouse testis mRNA and from a mixture of brain, liver, and kidney of rat mRNA (Rat B.L.K) and mouse (Mouse B.L.K) hybridized with htMART cDNA, washed under low stringency conditions, and exposed for 1 night. The size of the markers is indicated on the left hand side.

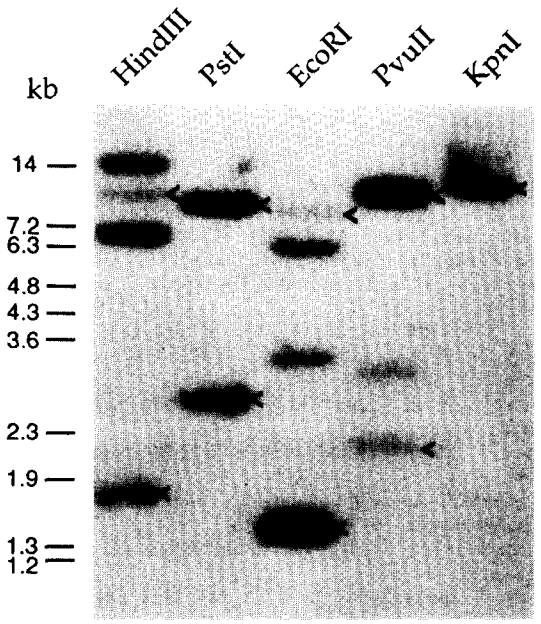

Fig. 4. Southern blot of human genomic DNA, cut with the indicated restriction enzymes and hybridized with htMART cDNA. The blot was washed under low stringency conditions and exposed for 1 night. The size of the markers is indicated on the left hand side. (<) indicates the bands which are still detected after a high stringency washing of the blot.

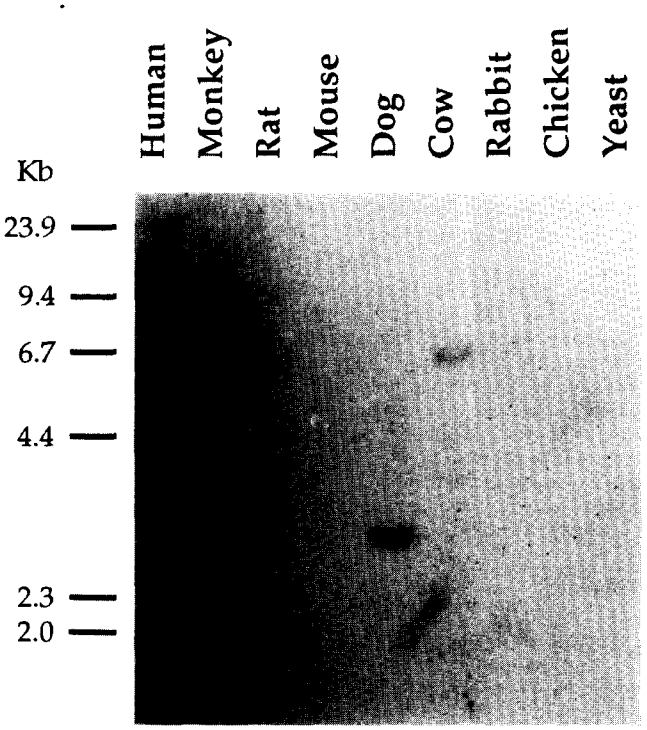

Fig. 5. Southern blot of genomic DNA from different species, cut with *Eco*RI, hybridized with htMART cDNA, washed under low stringency washing conditions and exposed for 5 nights. The size of the markers is indicated on the left hand side.

neither the RT6 specific for rodent T cell, nor the htMART expressed in human testis have their respective counterparts in the other species.

Initial attempts have been made in order to identify a human gene susceptible to encode for a rat RT6 homologue [23]. Using a probe, derived from the conserved consensus regions I and II (see Fig. 1), these authors cloned a human genomic sequence which exhibited around 80% identities with rat and mouse RT6, whereas we observed only 34% identities of this sequence with the predicted htMART protein. In addition this genomic sequence has three in frame stop codons, indicating that it corresponds to a non-transcribed pseudogene [23]. During the course of this cloning procedure, no genomic sequences related to htMART were isolated, although the initial primers were chosen in a very conserved region among species [23]. In addition, this human RT6 pseudogene is located in 11q13 [24], whereas we localized the htMART gene in 4q13-q21. Therefore it is clear that this pseudogene is more closely related to rodent RT6 than to htMART.

However, htMART is apparently homologous to other genes. This hypothesis is based on three observations: (i) under low stringency washing conditions, the htMART probe cross reacts with other transcripts in skeletal and heart muscle; (ii) in Southern blot of human genomic DNA, several bands are detected under low stringency hybridization conditions, in addition to the band specific for the htMART gene; (iii) the hybridization of htMART cDNA to genomic DNA isolated from different species, reveals a significant cross reactivity with monkey DNA and to a lesser extent with dog and cow and no signal on other sources including rat and mouse.

Thus it is clear that, at the genomic DNA level, the htMART and the rodent RT6 gene are not related. Although these genes might have a common ancestor, the rodent RT6 gene family is now represented by a pseudogene in human, whereas the htMART gene which has related sequence in monkey, dog and cow, does not have a rodent counterpart. Thus, both at the RNA and the DNA level, there is no more cross hybridization among those genes between human and rat. These observations demonstrate that only the partial sequencing associated with sequence comparison was able to establish a link among those genes. This is an efficient way to characterize new human genes as done for the human isologs of yeast suiI [25] and CDC10 [26] or for human genes involved in genetic diseases such as hereditary colon cancer [27] or X-linked glycerol kinase deficiency [28].

In conclusion, it is likely that htMART is not expressed in human lymphocyte and that it represents a new mono ADP-

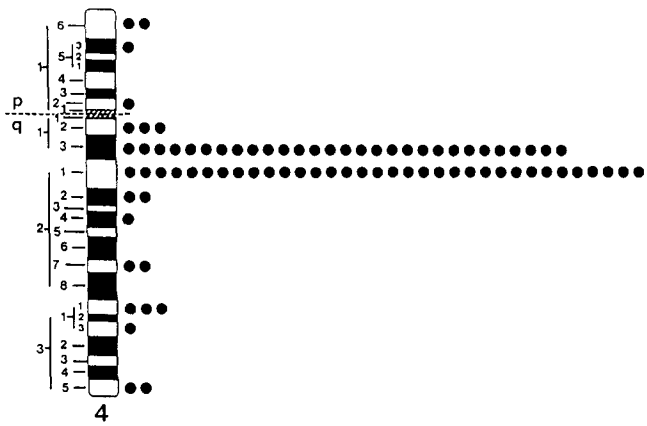

Fig. 6. Idiogram of the human G-banded chromosome 4 illustrating the distribution of labelled sites for the htMART probe.

ribosyl transferase specific from human testis. Until now, only one investigation has been found in the literature on the role of mono ADP-ribosylation in the testis, showing that the gonadotropin–testosterone system controls mono ADP-ribosylation of histone H2B and H3 in testis [29]. Thus, the transcript that we have described is of potential interest for the investigation of a new signal transduction pathway involving ADP-ribosylation in testis.

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References

[1] Koch, F., Haag, F., Kashan, A. and Thiele, H.G. (1990) Proc. Natl. Acad. Sci. 87, 964–967.
[2] Koch, F., Thiele, H.G. and Low, M.G. (1986) J. Exp. Med. 164, 1338–1343.
[3] Zolkiewska, A., Nightingale, M.S. and Moss, J. (1992) Proc. Natl. Acad. Sci. 89, 11352–11356.
[4] Haag, F., Andresen, V., Karsten, S., Koch-Nolte, F. and Thiele H.G. (1995) Eur. J. Immunol. 25, 2355–2361.
[5] Takada, T., Iida, K. and Moss, J. (1994) J. Biol. Chem. 269, 9420–9423.
[6] Greiner, D.L., Handler, E.S., Nakano, K., Mordes, J.P. and Rossini, A.A. (1986) J. Immunol. 136, 148–151.
[7] Prochazka, M., Gaskins, H.R., Leiter, E.H., Koch-Nolte, F., Haag, F. and Thiele, H.G. (1991) Immunogenetics 33, 152–156.
[8] Burstein, D., Mordes, J.P., Greiner, D.L., Stein, D., Nakamura, N., Handler, E.S. and Rossini, A.A. (1989) Diabetes 38, 24–30.
[9] Fowell, D. and Masson, D. (1993) J. Exp. Med. 177, 627–636.
[10] Koch-Nolte, F., Klein, J., Hollman, C., Kühl, M., Haag, F., Gaskins, H.R., Leiter, E. and Thiele H.G. (1994) Int. Immunol. 7, 883–890.
[11] Fangman, J., Schwinzer, R., Hedrich, H.J., Klöting, I. and Wonigeit, K. (1991) Eur. J. Immunol. 21, 2011–2013.
[12] Crisà, L., Sarkar, P., Waite, D.J., Haag, F., Koch-Nolte, F., Rajan, T.V., Mordes, J.P., Handler, E.S., Thiele, H.G., Rossini, A.A. and Greiner, D.L. (1993) Diabetes 42, 688–695.
[13] Crisa, L., Greiner, D.L., Mordes, J.P., MacDonald, R.G., Handler, E.S., Czech, M.P. and Rossini, A.A. (1990) Diabetes 39, 1279–1288.
[14] Pawlak, A, Toussaint, C., Lévy, I., Bulle, F., Poyard, M., Barouki, R. and Guellaen, G. (1995) Genomics 26, 151–158.
[15] Mattei, M.G., Philip, N., Passage, E., Moisan, J.P., Mandel, J.L. and Mattei, J.F. (1985) Hum. Genet. 69, 268–271.
[16] Takada, T., Iida, K. and Moss, J. (1995). J. Biol. Chem. 270, 541–544.
[17] Kyte A.M. and Doolitle, F. (1982) J. Mol. Biol. 157, 105–132.
[18] Ferguson, M.A.J. and Williams, A.F. (1988) Annu. Rev. Biochem. 57, 285–320.
[19] Haag, F., Koch, F. and Thiele, H.G. (1990) Nucl. Acid Res. 18, 1047.
[20] Koch, F., Haag, F. and Thiele, H.G. (1990) Nucl. Acid Res. 18, 3636.
[21] Zolkiewska, A., Nightingale, M.S. and Moss, J. (1992) Proc. Natl. Acad. Sci. 89, 11352–11356.
[22] Koch, F., Haag, F., Kashan, A. and Thiele, H.G. (1989) Immunology 67, 344–350.
[23] Haag, F., Koch-Nolte, F., Külh, M., Lorenzen, S. and Thiele, H.G. (1994) J. Mol. Biol. 243, 537–546.
[24] Koch-Nolte, F., Haag, F., Kühl, M., van Heyningen, V., Hoovers, J., Grzeschik, K.H., Singh, S. and Thiele, H.G. (1993) Genomics 18, 404–406.
[25] Fields C. and Adams, M.D. (1994) Biochem. Biophys. Res. Comm. 198, 288–291.
[26] Nakatsuru, S., Sudo, K. and Nakamura, Y. (1994) Biochem. Biohys. Res. Comm. 202, 82–87.
[27] Papadopoulos, N., Nikolaides, N.C., Wei, Y.F., Rubern, S.M., Carter, K.C., Rosen, C.A., Haseltine, W.A. et al. (1994) Science 263, 1625–1629.
[28] Sargent, C.A., Affara, N., Bentley, E., Pelmear, A., Bailey, D.M.D., Davey, P., Dow, D. et al. (1992) Hum. Mol. Genet. 2, 97–106.
[29] Kurokawa, T., Fujimura, Y., Takahashi, K., Chono, E., and Ishibashi, S. (1995) Biochem. Biophys. Res. Comm. 215, 808–813.]